"CLINICAL VALUE OF NON INVASIVE ASSESSMENT OF ENDOTHELIAL FUNCTION IN THE MANAGEMENT OF CORONARY ARTERY DISEASE"

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CERTIFICATE

This is to certify that the dissertation entitled **`CLINICAL VALUE OF NON INVASIVE ASSESSMENT OF ENDOTHELIAL FUNCTION IN THE MANAGEMENT OF CORONARY ARTERY DISEASE'** is the bonafide original work of **DR.M.KATHIRESAN** in partial fulfillment of the requirements for D.M. Branch-II (CARDIOLOGY) examination of THE TAMILNADU DR.M.G.R. MEDICAL UNIVERSITY to be held in August 2008.The period of post-graduate study and training was from August 2005 to July 2008.

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DECLARATION

I **Dr.M.KATHIRESAN,** solemnly declare that this dissertation entitled, **"CLINICAL VALUE OF NON INVASIVE ASSESSMENT OF ENDOTHELIAL FUNCTION IN THE MANAGEMENT OF CORONARY ARTERY DISEASE"** is a bonafide work done by me at the department of Cardiology, Madras Medical College and Government General Hospital during the period 2005 – 2008 under the guidance and supervision of the Professor and Head of the department of Cardiology of Madras Medical College and Government General Hospital, Professor R.Alagesan M.D.D.M. This dissertation is submitted to The Tamil Nadu Dr.M.G.R Medical University, towards partial fulfillment of requirement for the award of **D.M. Degree (Branch-II) in Cardiology.**

Place : Chennai

Date:

Dr. M.KATHIRESAN.

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CONTENTS

"learn to heal"

INTRODUCTION

Atherosclerosis begins in childhood, progresses silently through a long preclinical stage, and eventually manifests clinically, usually from middle age. Over the last 30 years, it has become clear that the initiation and progression of disease, and its later activation to increase the risk of morbid events, depends on profound dynamic changes in vascular biology[**1**]. The endothelium has emerged as the key regulator of vascular homeostasis, in that it has not merely a barrier function but also acts as an active signal transducer for circulating influences that modify the vessel wall phenotype. Alteration in endothelial function precedes the development of morphological atherosclerotic changes and can also contribute to lesion development and later clinical complications[**2**].

The vascular endothelium is a large paracrine organ that secretes numerous factors regulating vascular tone, cell growth, platelet and leukocyte interactions and thrombogenicity.The endothelium senses and responds to a myriad of internal and external stimuli through complex cell membrane receptors and signal transduction mechanisms, leading to the synthesis and release of various vasoactive,thromboregulatory and growth factor substances. Endothelial dysfunction is thought to be an important factor in the development of atherosclerosis, hypertension, and heart failure.

A disturbance of endothelial function is considered as a key event in the development of atherosclerosis . Thus reliable assessment of endothelial function in humans appears highly desirable. With respect to the major endothelial functions, this aim can be achieved by different approaches:

1. measurement of morphological and mechanical characteristics of the vascular wall (intima media thickness, compliance, distensibility, and remodelling indexes);

2. determination of soluble endothelial markers (von Willebrandt factor, plasminogen activator, inhibitor complex thrombomodulin adhesion molecules, and *N*-oxides); and

3. measurement of the endothelium-dependent regulation of vascular tone at

focal sites of the circulation.

The endothelium is of essential importance for the maintenance of vascular tone. It participates in the regulation of blood flow in response to changes in tissue and organ perfusion requirements. When blood flow increases through a vessel, the vessel dilates. This phenomenon has been coined flow-mediated dilatation (FMD).[**3**] Schretzenmayer was first to describe this physiological response, and FMD has been demonstrated subsequently in a number of conduit arteries in vitro and in vivo, in animals and in humans[**4**].

The effect of disease states and/or interventions on the blood flow response to cuff occlusion (reactive hyperemia) is underexplored. Current technology limits the utility of spectral Doppler to reproducibly assess changes in flow, which might provide useful information about endothelial function of the microvasculature. Tremendous interest exists in determining the clinical utility of brachial artery FMD. Investigators have hypothesized that endothelial function may serve as an integrating index of risk factor burden and genetic susceptibility, and that endothelial dysfunction will prove to be a preclinical marker of cardiovascular disease

Several studies suggest that the presence of endothelial dysfunction in the coronary circulation is an independent predictor of cardiovascular disease events. The technique is particularly well suited for study of the earliest stages of atherosclerosis in children and young adults, thus providing maximal opportunity for prevention. Numerous studies have demonstrated that brachial artery reactivity improves with risk factor modification and treatment with drugs known to reduce cardiovascular risk. It remains unknown whether an improvement in endothelial function directly translates into improved outcome. In the future, however, practitioners may use brachial artery FMD to assess response to drug therapy and to individualize patient risk factor modification programs. Further studies are needed to determine whether the methodology is sufficiently reproducible and whether biological variability is sufficiently low to make assessment of FMD a clinically useful measure of cardiovascular risk on an individual or group basis[**5**]

Measurement of endothelial function in patients has recently emerged as a useful tool for atherosclerosis research. In the setting of cardiovascular disease (CVD) risk factors, the endothelium loses its normal regulatory functions. Clinical syndromes such as stable and unstable angina, acute myocardial infarction, claudication, and stroke relate, in part, to a loss of endothelial control of vascular tone, thrombosis, and the composition of the vascular wall. Recent studies have shown that the severity of endothelial dysfunction relates to the risk for an initial or recurrent cardiovascular event. Finally, a growing number of interventions known to reduce cardiovascular risk also improve endothelial function. This concept has prompted speculation that endothelial function serves as a "barometer" for cardiovascular health that can be used for patient care and evaluation of new therapeutic strategies

Assessment of conventional risk factor burden is necessary but may not accurately estimate risk of cardiovascular disease[**6**] Most patients in whom myocardial infarction or ischemic stroke develops have one or more conventional risk factors for atherosclerosis, but these risk factors are also prevalent in the general population. As a result, the predictive value of algorithms based on conventional risk factors is unsatisfactory[**7, 8**]. Nearly 40% of adults presenting to cardiology op may be at intermediate risk for a future cardiovascular event when assessed with current algorithms and these individuals may benefit from further risk stratification. The available screening and diagnostic tests have limitations; cardiac stress tests detect only advanced, hemodynamically significant lesions, and conventional coronary angiography is invasive, provides only a "luminogram," and does not identify vulnerable or unstable plaque. Tests for early detection of atherosclerotic vascular disease are therefore needed to better assess cardiovascular risk in asymptomatic individuals, the main focus of primary prevention.

Noninvasive arterial testing for cardiovascular risk assessment is based on several important considerations. Alterations in arterial function and structure predate clinical manifestations of occlusive atherosclerotic disease; changes tend to be widespread and are not limited to a single arterial bed. These alterations result from

the cumulative effects of known and unknown vascular risk factors that promote formation and progression of atherosclerotic lesions and may also increase the propensity for atherosclerotic plaque rupture . Identification of such abnormalities in accessible peripheral arteries provides a means for early detection of presymptomatic vascular disease and improved cardiovascular risk stratification.

Arterial ultrasonography and tonometry are attractive modalities for detecting early disease because they are noninvasive and relatively inexpensive. High-resolution ultrasonography can assess arterial dilatation in response to shear stress or pharmacological stimuli (a function that may be compromised early in atherogenesis) and directly examine the arterial wall for early atherosclerotic changes preceding luminal compromise. Arterial tonometry can be used to acquire arterial pulse waveforms to assess arterial stiffness and wave reflection, measures that have been associated with the presence and extent of atherosclerotic vascular disease and cardiovascular events.

AIM OF THE STUDY

Most patients in whom myocardial infarction or ischemic stroke develops have one or more conventional risk factors for atherosclerosis, but these risk factors are also prevalent in the general population. As a result, the predictive value of current algorithms based on conventional risk factors is unsatisfactory.

There is growing evidence that endothelial function serves as a “barometer” for cardiovascular health and assessment of endothelial function will further help in refining risk in an individual patient and therefore guide in intensity of management.With this background, aimed to study

1. To assess the incremental value of non invasive assessment of endothelial function by flow mediated dilatation of brachial artery and aortic pulse wave velocity in risk stratification and management of low and intermediate risk patients

2. To assess the feasibility of inclusion of flow mediated dilatation and aortic pulse wave velocity in routine cardiac clinical practice

REVIEW OF LITERATURE

ENDOTHELIUM IN NORMAL VASCULAR HOMEOSTASIS

Although only a simple monolayer, the healthy endothelium is optimally placed and is able to respond to physical and chemical signals by production of a wide range of factors that regulate vascular tone, cellular adhesion, thromboresistance, smooth muscle cell proliferation, and vessel wall inflammation. The importance of the endothelium was first recognized by its effect on vascular tone. This is achieved by production and release of several vasoactive molecules that relax or constrict the vessel, as well as by response to and modification of circulating vasoactive mediators such as bradykinin and thrombin. This vasomotion plays a direct role in the balance of tissue oxygen supply and metabolic demand by regulation of vessel tone and diameter, and is also involved in the remodeling of vascular structure and long-term organ perfusion[**9**].

The pioneering experiments of Furchgott and Zawadzki first demonstrated an endothelium-derived relaxing factor that was subsequently shown to be nitric oxide[**10**] . NO is generated from L-arginine by the action of endothelial NO synthase (eNOS) in the presence of cofactors such as tetrahydrobiopterin. This gas diffuses to the vascular smooth muscle cells and activates guanylate cyclase, which leads to cGMP-mediated vasodilatation. Shear stress is a key activator of eNOS in normal physiology, and this adapts organ perfusion to changes in cardiac output. In addition, the enzyme may be activated by signaling molecules such as bradykinin, adenosine, vascular endothelial growth factor (in response to hypoxia), and serotonin (released during platelet aggregation). The endothelium also mediates hyperpolarization of vascular smooth muscle cells via an NO-independent pathway, which increases potassium conductance and subsequent propagation of depolarization of vascular smooth muscle cells, to maintain vasodilator tone. The endothelium-derived hyperpolarizing factors involved in this process are only partially understood (such as the cytochrome-derived factors and possibly C-type natriuretic peptide), and may differ between vascular beds. However, it is well recognized that Endothelium-Derived Hyperpolarizing Factor can compensate for loss of NO-mediated vasodilator tone, particularly in the microcirculation, and this appears important when NO bioavailability is reduced[**11**].

Prostacyclin, derived by the action of the cyclooxygenase system, is another endothelium-derived vasodilator that acts independently of NO. Although it may contribute to some of the other regulatory roles of the endothelium, it appears to have a more limited role in the maintenance of vasodilator tone in humans.

The endothelium modulates vasomotion, not only by release of vasodilator substances, but also by an increase in constrictor tone via generation of endothelin and vasoconstrictor prostanoids, as well as via conversion of angiotensin I to angiotensin II at the endothelial surface[**12, 13**]. These vasoconstrictor agents predominantly act locally, but may also exert some systemic effects and have a role in the regulation of arterial structure and remodeling.

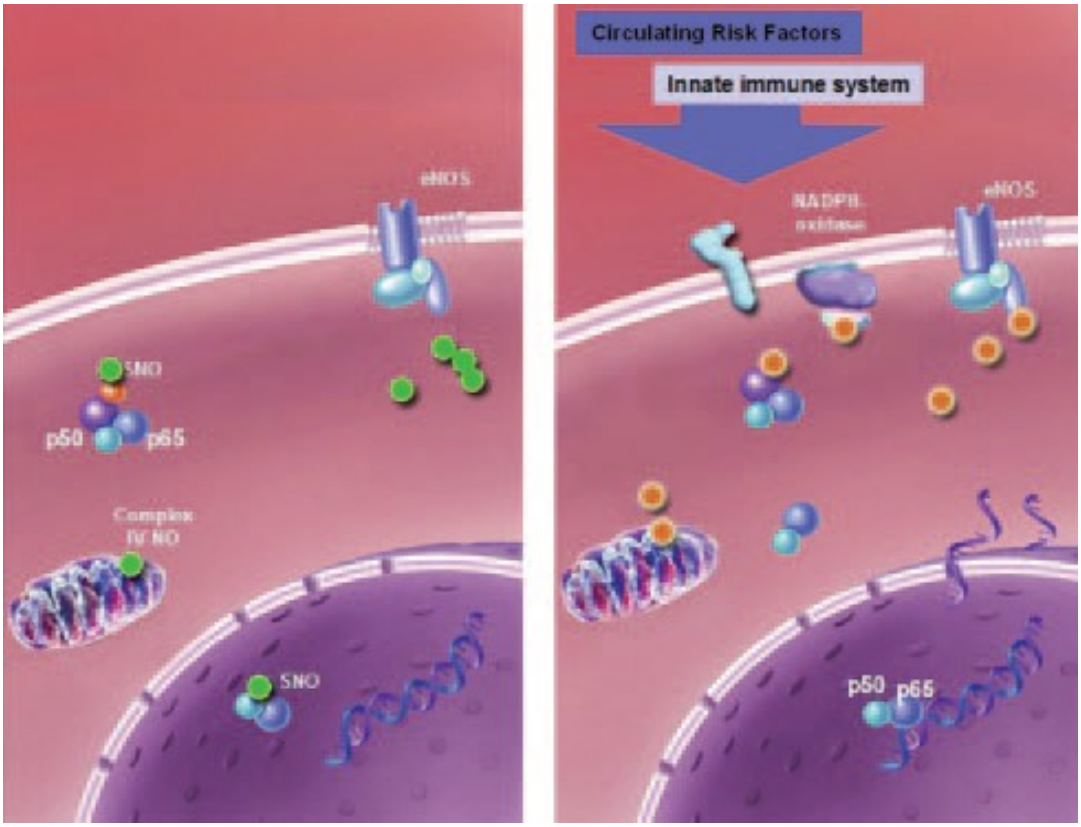


Figure 1. Left, The quiescent state of the endothelium, where NO (green circles) is generated by the endothelial isoform of nitric oxide synthase (eNOS) in its membrane-bound configuration. The released NO targets cysteine groups in key regulator molecules such as NF_B (p50/p65) and the mitochondria, which leads to silencing of cellular processes. Right, The state of endothelial activation where reactive oxygen signaling (red circles) predominates. The ROS such as H2O2 are generated from oxidases as well as the uncoupled state of eNOS. Like NO, the molecules target key regulatory proteins, such as NF_B and phosphatases, which leads to activation of the endothelial cells. Such activation can occur Physiologically in the context of host defense or pathophysiologically in the presence of cardiovascular risk factors. EC indicates endothelial cells.

PATHOPHYSIOLOGY OF ENDOTHELIAL DYSFUNCTION

ENDOTHELIAL ACTIVATION AND ATHEROSCLEROSIS

What is generally referred to as endothelial dysfunction should more appropriately be considered as endothelial activation, which may eventually contribute to arterial disease when certain conditions are fulfilled. Endothelial activation represents a switch from a quiescent phenotype toward one that involves the host defense response . Indeed, most cardiovascular risk factors activate molecular machinery in the endothelium that results in expression of chemokines, cytokines, and adhesion molecules designed to interact with leukocytes and platelets and target inflammation to specific tissues to clear microorganisms.

The fundamental change involved in this process is a switch in signaling from an NO-mediated silencing of cellular processes toward activation by redox signaling. Reactive oxygen species (ROS), in the presence of superoxide dismutase, lead to generation of hydrogen peroxide, which, like NO, can diffuse rapidly throughout the cell and react with cysteine groups in proteins to alter their function. However, because of the different chemistry involved, this results in very different consequences, such as phosphorylation of transcription factors, induction of nuclear chromatin remodeling and transcription genes, and protease activation. It is intriguing that eNOS, which normally helps maintain the quiescent state of the endothelium, can switch to generate ROS in appropriate circumstances as part of endothelial activation. This is termed eNOS uncoupling, and results in superoxide formation if the key cofactor tetrahydrobiopterin is not present, or generation of hydrogen peroxide if the substrate L-arginine is deficient. Thus, the ability of eNOS to regulate both the quiescent and activated endothelial phenotype puts this enzyme at the center of endothelial homeostasis[**17, 18**].

If endothelial activation and redox signaling are part of normal host defense, it is intriguing to consider the circumstances in which they may contribute to atherogenesis and clinical events. The difference between normal host defense and detrimental cellular activation may well be a consequence of the nature, extent, duration, and combination of the proinflammatory stimuli. For example, recent studies have shown a profound but transient reduction of endothelium dependent

dilatation associated with mild childhood infection. This may be adaptive and not necessarily proatherogenic, but could become so if other adverse environmental conditions are also present. These might include risk factors such as hypercholesterolemia, hypertension, and diabetes, as well as other inflammatory conditions, such as periodontitis, which may induce chronic dysregulation of NO and ROS production. All of these environmentally driven mechanisms of endothelial activation are likely to be modulated by genetic factors[**19**].

In certain circumstances, chronic production of ROS may exceed the capacity of cellular enzymatic and nonenzymatic anti-oxidants, and thus contribute to vascular disease by induction of sustained endothelial activation.

An important source of ROS is probably the mitochondrion, in which production of ROS and the dismuting capacity of mitochondrial superoxide dismutase are typically carefully balanced during oxidative phosphorylation. This may be disturbed during hypoxia or conditions of increased substrate delivery, such as occurs in obesity-related metabolic disorders or type II diabetes, which are characterized by hyperglycemia and increased circulating free fatty acids. Other important sources of oxidative stress in the endothelium are nicotinamide adenine dinucleotide phosphate oxidases, as well as xanthine oxidase, which have been shown to have increased activity in arteries from patients with coronary disease. Endothelial ROS signaling may be initiated by exposure to inflammatory cytokines and growth factors, and the interaction of the endothelium with leukocytes.

Regardless of their source, the interaction between ROS and NO sets up a vicious circle, which results in further endothelial activation and inflammation[**20**].

ENDOTHELIAL INJURY AND REPAIR

Prolonged and/or repeated exposure to cardiovascular risk factors can ultimately exhaust the protective effect of endog-enous antiinflammatory systems within endothelial cells. As a consequence, the endothelium not only becomes dysfunctional, but endothelial cells can also lose integrity, progress to senescence, and detach into the circulation. Circulating markers of such endothelial cell damage include endothelial microparticles derived from activated or apoptotic cells, and

whole endothelial cells. These markers have been found to be increased in both peripheral and coronary atherosclerosis disease, as well as other inflammatory conditions associated with increased vascular risk such as rheumatoid arthritis and systemic lupus erythematosus. Circulating endothelial microparticles and endothelial cells can be quantified, and may be promising candidates for clinical testing.

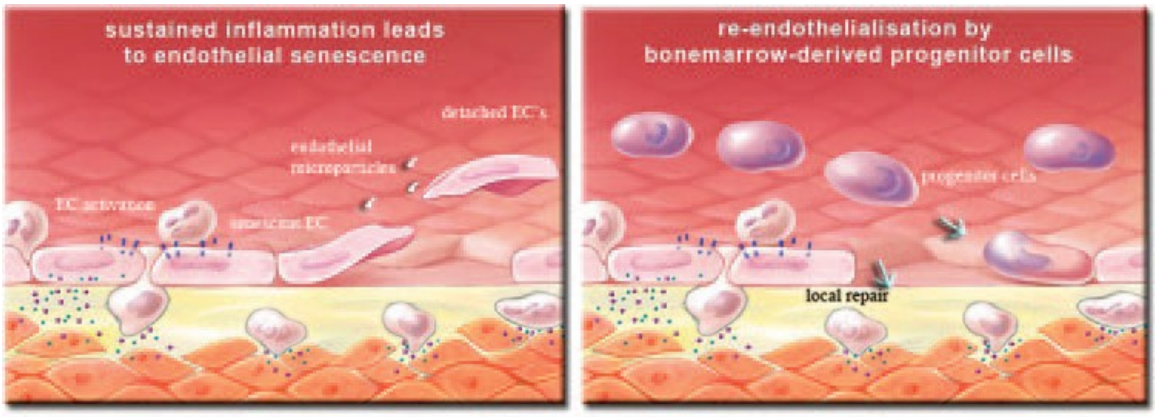


Figure 2. Sustained ROS signaling induces senescence of endothelial cells. Left, This is reflected in detachment of endothelial cells or parts of the endothelial cell membrane (endothelial microparticles). Right, With increasing age and persisting ROS signaling, the capacity of neighboring endothelial cells to repair the endothelial injury is limited, and vascular integrity becomes dependent on the incorporation of circulating progenitor cells

Endothelial integrity depends not only on the extent of injury, but also on the endogenous capacity for repair. Two mechanisms by which this process of repair occurs have been recently identified. Adjacent mature endothelial cells can replicate locally, and replace the lost and damaged cells. A recent modeling study suggested that, although local endothelial cells would be sufficient to maintain vascular integrity throughout life in healthy circumstances, in the presence of risk factors, loss of endothelial integrity would rapidly develop if local replication were the only repair mechanism. More recently, it has become clear that circulating endothelial progenitor cells are an alternative mechanism for maintenance and repair of the endothelium. These cells are recruited from the bone marrow, circulate in the peripheral blood, and can differentiate into mature cells with endothelial characteristics. Mobilization of these cells is in part NO dependent, and may thus be impaired in patients with cardiovascular risk factors.

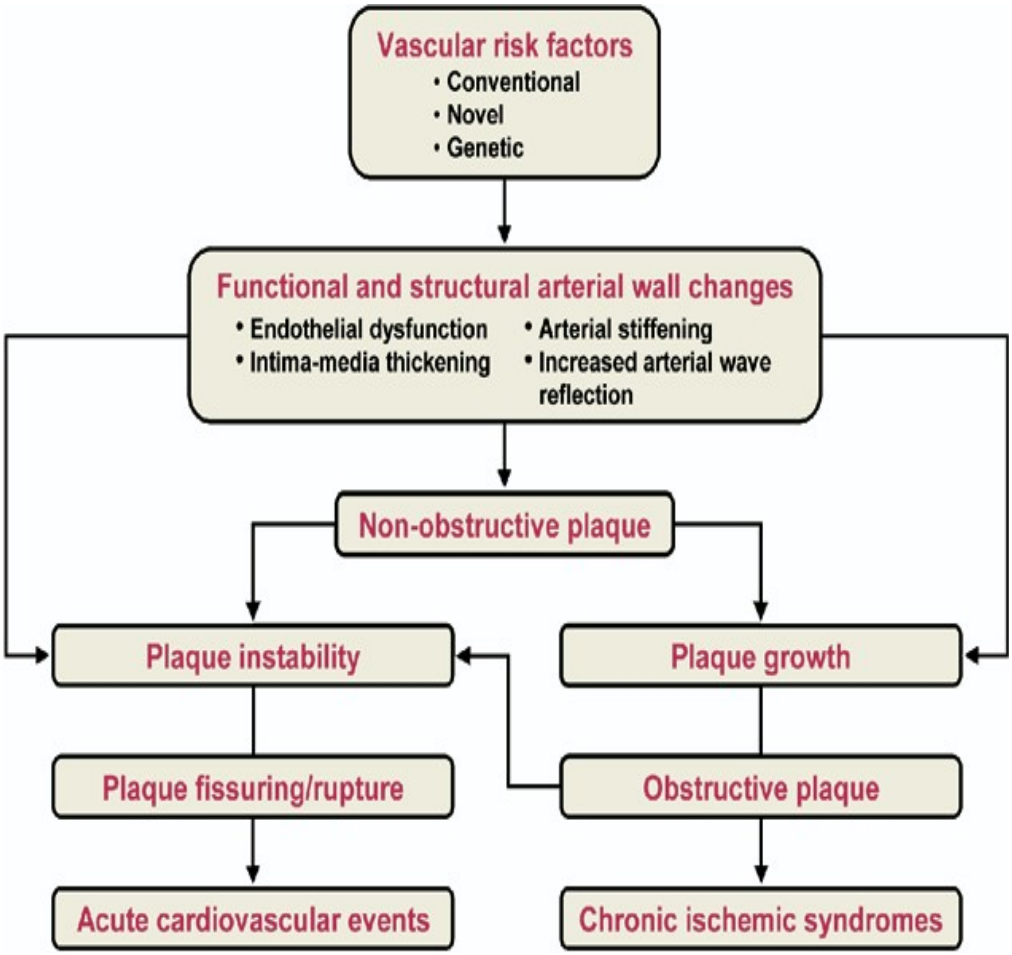
Conversely, factors that have been shown to improve endothelial function and NO bioavailability, such as exercise and statins, have also been shown to have a potent positive effect on endothelial progenitor cell mobilization. Furthermore, recent evidence has indicated that risk factors not only interfere with the recruitment of circulating endothelial progenitor cells, but also with the differentiation and function of these cells. For example, important cellular properties such as migration, adhesion, and formation of tubules in culture conditions can be impaired in the presence of risk factors and atherosclerotic disease. It is intriguing to note that circulating endothelial progenitor cells may differentiate down different lineages, and develop characteristics of other myeloid cells such as macrophages and dendritic cells when exposed to inflammatory cytokine profiles. Thus, circulating endothelial progenitor cell biology may play a major role in the pathogenesis of vascular disease by an effect on both endothelial injury and the capacity for endothelial repair. The importance of the balance between exposure to risk factors and the capacity for repair in the determination of the clinical endothelial phenotype has been highlighted by the demonstration that subjects with increased numbers of circulating endothelial progenitor cells have preserved endothelial function, despite exposure to high levels of risk factors[**21, 22, 23, 24**].

CLINICAL ASSESSMENT OF ENDOTHELIAL FUNCTION

An improved understanding of the vascular biology of the endothelium has permitted the development of clinical tests that evaluate several of the functional properties of normal and activated endothelium. Ideally, such tests should be safe, noninvasive, reproducible, repeatable, cheap, and standardized between laboratories. The results should also reflect the dynamic biology of the endothelium throughout the natural history of atherosclerotic disease, define subclinical disease processes, as well as provide prognostic information for risk stratification in the later clinical phase. No single test currently fulfils these requirements, and a panel of several tests is therefore needed to characterize the multiple facets of endothelial biology.

The currently available modalities are

1. Cardiac catheterization [change in diameter, change in coronary blood flow]
2. Venous occlusion plethysmography[change in forearm blood flow]
3. Flow mediated dilatation[change in brachial artery diameter]
4. Aortic pulse wave velocity[change in pulse amplitude]
5. Pulse wave analysis[change in augmentation index]
6. Pulse contour analysis[change in reflective index] [**25**]



Association of Altered Arterial Function and Structure With Cardiovascular Events

FLOW MEDIATED DILATATION

MECHANISMS AND PHYSIOLOGICAL ASPECTS

The capacity of blood vessels to respond to physical and chemical stimuli in the lumen confers the ability to self regulate tone and to adjust blood flow and distribution in response to changes in the local environment. Many blood vessels respond to an increase in flow, or more precisely shear stress, by dilating. This phenomenon is designated *FMD*. A principal mediator of FMD is endothelium derived NO.

The precise mechanisms for the acute detection of shear forces and subsequent signal transduction to modulate vasomotor tone are not fully understood. The endothelial cell membrane contains specialized ion channels, such as calcium-activated potassium channels, that open in response to shear stress . The effect of potassium channel opening is to hyperpolarize the endothelial cell, increasing the driving force for calcium entry (there are no voltage gated calcium channels in endothelial cells). Calcium activates an enzyme, endothelial nitric oxide synthase (eNOS), and the subsequent generation of NO appears to account for FMD . Indeed, endothelial denudation or treatment with a nitric oxide synthase (NOS) inhibitor abolishes FMD in a variety of arterial vessels. However, it was recently shown that blood vessels from mice genetically engineered to lack the eNOS enzyme (eNOS knockout mice) still respond to shear stress by dilating . In the eNOS knockout mice, FMD seems to be mediated by endothelium-derived prostanoids, as it is blocked by indomethacin . Thus, there is some redundancy in the system, and more than one endothelial mediator is capable of acting as the signal between endothelium and smooth muscle. It is unknown whether other mediators, such as the putative endothelium-derived hyperpolarizing factor, can cause FMD if both NO and prostanoids are deficient [**26, 27, 28**].

Several mechanisms may underlie the increase in NO in response to changes in shear stress. Very acute changes may be mediated by the increase in intracellular calcium that occurs when ion channels open . Over slightly longer time periods (minutes), shear-stress-induced phosphorylation of eNOS via a serine/threonine protein kinase, Akt/PKB, increases eNOS activity, even at low calcium

concentrations, and this may be important to allow continued output of NO . In addition, other posttranslational modifications of the enzyme (myristilation or palmitoylation) or interaction with caveolin can affect intracellular localization of the enzyme and thereby alter its function. Over longer time periods (many minutes or hours), eNOS gene transcription is activated, and this can result in continued increases in NO generation if shear stress is maintained at high levels [**29, 30**].

FMD; TECHNICAL ASPECTS

SUBJECT PREPARATION.

Numerous factors affect flow mediated vascular reactivity, including temperature, food, drugs and sympathetic stimuli, among others. Therefore, subjects should fast for at least 8 to 12 h before the study, and they should be studied in a quiet, temperature controlled room. All vasoactive medications should be withheld for at least four half-lives, if possible. In addition, subjects should not exercise, should not ingest substances that might affect FMD such as caffeine, high-fat foods and vitamin C or use tobacco for at least 4 to 6 h before the study. The investigator should be cognizant of the phase of the subject's menstrual cycle, as it too may affect FMD . All of these confounding factors must be considered in preparing a subject in studies that seek to determine the impact of a single intervention. For observational cohort studies, data must be collected on those factors known to affect the measurement of FMD, and analysis should address their impact .

THE ULTRASOUND SYSTEM

Ultrasound systems must be equipped with vascular software for two-dimensional (2D) imaging, color and spectral Doppler, an internal electrocardiogram (ECG) monitor and a high-frequency vascular transducer. A linear array transducer with a minimum frequency of 7 MHz, attached to a high-quality mainframe ultrasound system, is used to acquire images with sufficient resolution for subsequent analysis. Image resolution is enhanced with broadband (multiple-frequency: 7 to 12 MHz) linear array transducers. Timing of each image frame with respect to the cardiac cycle is determined with simultaneous ECG recording on the ultrasound system video monitor.

IMAGE ACQUISITION

The subject is positioned supine with the arm in a comfortable position for imaging the brachial artery. The brachial artery is imaged above the antecubital fossa in the longitudinal plane . A segment with clear anterior and posterior intimal interfaces between the lumen and vessel wall is selected for continuous 2D grayscale imaging. Currently, cross-sectional imaging of the brachial artery cannot be used to determine maximum diameter or area of the lumen because of inadequate image definition of the lateral walls. Also, skew artifacts from cross-sectional imaging limit accurate diameter determination. In addition to 2D grayscale imaging, both M mode and A mode (wall tracking) can be used to continuously measure the diameter , yet these techniques may be more subject to error owing to tracking drift. No direct comparison has been made of diameter determinations from continuous recording using grayscale images versus wall tracking. During image acquisition, anatomic landmarks such as veins and fascial planes are noted to help maintain the same image of the artery throughout the study. A stereotactic probe-holding device can be helpful [**31, 32**].

ENDOTHELIUM DEPENDENT FMD.

To create a flow stimulus in the brachial artery, a sphygmomanometric (blood pressure) cuff is first placed either above the antecubital fossa or on the forearm. A baseline rest image is acquired, and blood flow is estimated by time-averaging the pulsed Doppler velocity signal obtained from a midartery sample volume. Thereafter, arterial occlusion is created by cuff inflation to suprasystolic pressure. Typically, the cuff is inflated to at least 50 mm Hg above systolic pressure to occlude arterial inflow for a standardized length of time. This causes ischemia and consequent dilation of downstream resistance vessels via autoregulatory mechanisms. Subsequent cuff deflation induces a brief high-flow state through the brachial artery (reactive hyperemia) to accommodate the dilated resistance vessels. The resulting increase in shear stress causes the brachial artery to dilate. The longitudinal image of the artery is recorded continuously from 30 s before to 2 min after cuff deflation. A midartery pulsed Doppler signal is obtained upon immediate cuff release and no later than 15 s after cuff deflation to assess hyperemic velocity [**33**]

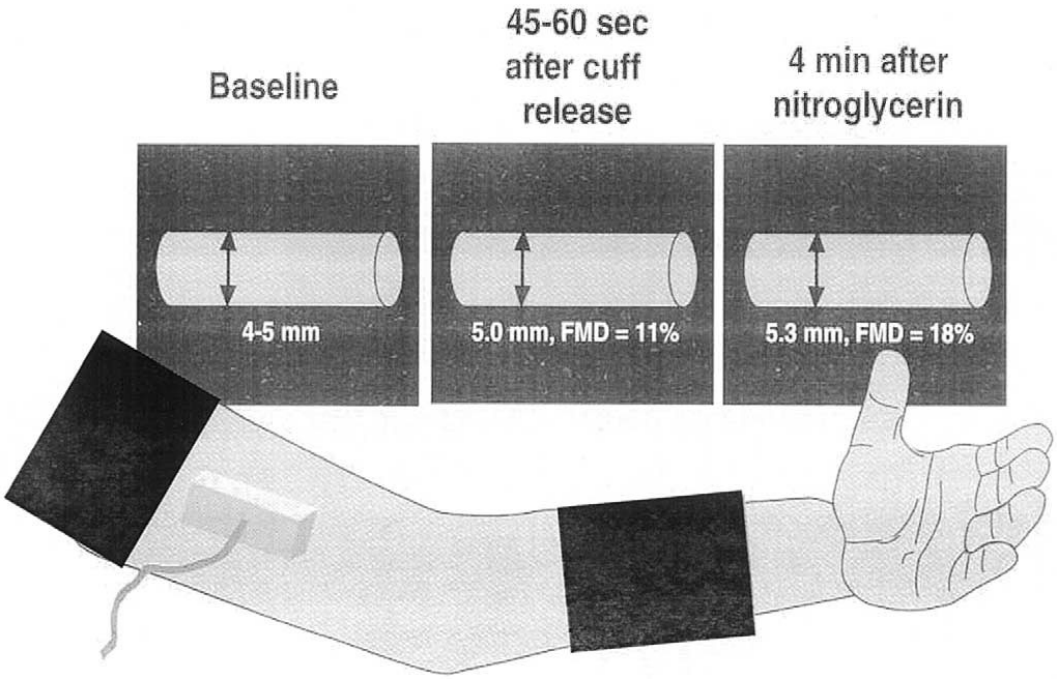


Figure 4 Schematic drawing of ultrasound imaging of the brachial artery with **upper** versus **lower cuff** placement and transducer position above the antecubital fossa. BP _ blood pressure; FMD _ flow-mediated vasodilation.

Studies have variably used either upper arm or forearm cuff occlusion, and there is no consensus as to which technique provides more accurate or precise information . When the cuff is placed on the upper part of the arm, reactive hyperemia typically elicits a greater percent change in diameter compared with that produced by the placement of the cuff on the forearm . This may be due to a greater flow stimulus resulting from recruitment of more resistance vessels or possibly to direct effects of ischemia on the brachial artery. However, upper-arm occlusion is technically more challenging for accurate data acquisition as the image is distorted by collapse of the brachial artery and shift in soft tissue. The change in brachial artery diameter after cuff release increases as the duration of cuff inflation increases from 30 s to 5 min. The change in diameter is similar after 5 and 10 min of occlusion; therefore, the more easily tolerated 5-min occlusion is typically used. Also, FMD may be studied in the radial, axillary and superficial femoral arteries. Notable caveats are that arteries smaller than 2.5 mm in diameter are difficult to measure, and vasodilation is generally less difficult to perceive in vessels larger than 5.0 mm in diameter [**34**].

ENDOTHELIUM INDEPENDENT DILATATION WITH NITROGLYCERIN.

At least 10 min of rest is needed after reactive hyperemia (i.e., FMD) before another image is acquired to reflect the reestablished baseline conditions. In most studies to date, an exogenous NO donor, such as a single high dose (0.4 mg) of nitroglycerin (NTG) spray or sublingual tablet has been given to determine the maximum obtainable vasodilator response, and to serve as a measure of endothelium-independent vasodilation reflecting vascular smooth muscle function. Peak vasodilation occurs 3 to 4 min after NTG administration; images should be continuously recorded during this time, and NTG should not be administered to individuals with clinically significant bradycardia or hypotension. Determining the vasodilator responses to increasing doses of NTG, rather than a single dose, may further elucidate changes in smooth muscle function or arterial compliance that might be playing a role in any observed changes in FMD [**35**].

FMD; ANALYSIS

ANATOMIC LANDMARKS.

The diameter of the brachial artery should be measured from longitudinal images in which the lumen-intima interface is visualized on the near (anterior) and far (posterior) walls). These boundaries are best visualized when the angle of insonation is perpendicular. Thus, clear visualization of both the near and far wall lumen-intima boundaries indicates that the imaging plane is bisecting the vessel in the longitudinal direction, and diameters measured from these images likely reflect the true diameter.

Once the image for analysis is chosen, the boundaries for diameter measurements (the lumen-intima or the media-adventitia interfaces) are identified manually with electronic calipers or automatically using edgedetection software. The variability of the diameter measurement is greatest when it is determined from a point-to point measurement of a single frame, and least when there is an average derived from multiple diameter measurements determined along a segment of the vessel. Similarly, cross-sectional images are less reliable, for only a single point in the vessel's length is used to determine maximal diameter. The diameter measurement along a longitudinal segment of vessel is dependent upon the alignment of the image.

Skew occurs when the artery is not completely bisected by the plane of the ultrasound beam. With slight skew, the maximal diameter measured is constant, and thus yields a more accurate measurement. Some edge-detection programs can account for skew from transducer angulation .

TIMING OF FMD.

Flow-mediated vasodilation is an endothelium-dependent process that reflects the relaxation of a conduit artery when exposed to increased shear stress. Increased flow, and thereby increased shear stress, through the brachial artery occurs during postocclusive reactive hyperemia. Several studies have suggested that the maximal increase in diameter occurs approximately 60 s after release of the occlusive cuff, or 45 to 60 s after peak reactive hyperemic blood flow . The increase in diameter at this time is prevented by the NOS inhibitor NG-monomethyl- L-arginine, indicating that it is an endothelium-dependent process mediated by NO . Other measures of vasodilator response include time to maximum response , duration of the vasodilator response and the area under the dilation curve .

TIMING DURING CARDIAC CYCLE.

Brachial artery diameter should be measured at the same time in the cardiac cycle, optimally achieved using ECG gating during image acquisition. The onset of the R-wave is used to identify end diastole, and the peak of the T-wave reproducibly identifies end systole. Peak systolic diameter is larger than end systolic diameter, because the vessel expands during systole to accommodate the increase in pressure and volume generated by left ventricular contraction. The magnitude of systolic expansion is affected by the vessel compliance, and it may be reduced by factors such as aging and hypertension (possibly by reduced bioavailability of NO). Thus, functional characteristics of the brachial artery may obfuscate the measurement of FMD if diameter is measured during end systole; however, this concern has not been tested in a rigorous trial.

CHARACTERIZING FMD.

Flow-mediated vasodilation is typically expressed as the change in post-stimulus diameter as a percentage of the baseline diameter . Baseline diameter influences percent change in two ways. First, for any given absolute change in the postflow stimulus diameter, a larger baseline diameter yields a smaller measure of percent change. Reporting absolute change in diameter will minimize this problem. Second, smaller arteries appear to dilate relatively more than do larger arteries . Both factors merit consideration when comparing vasodilator responses between individuals and groups with different baseline diameters. For studies in which comparisons are made before and after an intervention in the same individuals, percent change might be the easiest method to use if baseline diameter remains stable over time. However, the best policy may be to measure and report baseline diameter, absolute change and percent change in diameter.

FMD; IMPORTANT STUDIES AND OUTCOMES

Arterial vasodilatation in response to shear stress produced by increased flow is mediated predominantly by endothelium-derived nitric oxide , and hence, flow-mediated dilatation (FMD) is considered a biomarker of endothelial function .

Because endothelial dysfunction is associated with cardiovascular events, measurement of brachial artery FMD may help to identify patients at risk. The prognostic value of brachial artery FMD has been shown in several patient populations. *Chan et al.*[**36**] showed that in patients with coronary heart disease (CHD), impaired baseline FMD and its further decrease over time predicted the occurrence of adverse cardiovascular events. In patients with peripheral arterial disease, impaired FMD was predictive of early and late adverse cardiovascular events after vascular surgery, even after accounting for risk factors and the ankle-brachial index according to *Gokce N et al* [**37, 38**]. In patients undergoing evaluation for chest pain, impaired FMD was predictive of adverse cardiac events in the near term and long term[*Neunteufl T, Heher S, Katzenschlager R, et al*] [**39**]. Two recent studies first by *Meyer B, Mortl D, Strecker K, et al*.[**40**] and second by *Katz SD, Hryniewicz K, Hriljac I, et al.* [**41**] showed FMD to be predictive of increased mortality and the need for cardiac transplantation in patients with congestive heart failure.

Data about the incremental prognostic value of FMD in asymptomatic subjects are limited. In a study of 444 subjects at increased cardiovascular risk *Fathi R, Haluska B, Isbel N, Short L, Marwick TH et al* [**42]** showed, the incidence of adverse cardiovascular events and all-cause mortality over a median follow-up period of 2 years was significantly higher in subjects with FMD less than 2% compared with those with FMD more than 2%. However, FMD was not an independent predictor of these events after adjustment for conventional risk factors. Whether an improvement in FMD translates into improved clinical outcome is yet to be established, although in a study by *Modena MG, Bonetti L, Coppi F, Bursi F, Rossi R et al* [**43**] involving 400 hypertensive postmenopausal women, failure to improve FMD after 6 months of optimal antihypertensive therapy was associated with increased risk of nonfatal cardiovascular events over a mean follow-up of 67 months .

In addition to FMD, brachial artery ultrasound also provides a means of assessing other measures of arterial function that may be associated with cardiovascular risk. For example, impaired brachial artery dilatation to sublingually administered nitroglycerin, an "endothelium-independent" response that reflects arterial smooth muscle function , has been noted to be impaired in the presence of cardiovascular disease [*Zhang X, Zhao SP, Li XP, Gao M, Zhou QC.*] [**44**] as well as in asymptomatic subjects with risk factors [*Adams MR, Robinson J, McCredie R, et al*] [**45**]. and [*Yugar-Toledo JC, Tanus-Santos JE, Sabha M, et al*.] [**46**]. Brachial artery ultrasound can be combined with pulsed Doppler to measure forearm blood flow at rest and during the phase of hyperemia after transient forearm occlusion; both measures reflect microvascular function. Several cardiovascular risk factors have been found to be associated with higher resting forearm blood flow and a blunted reactive hyperemic response [*Higashi Y, Sasaki S, Nakagawa K, Matsuura H, Kajiyama G, Oshima Tet al*] [**47**]. Postischemic reactive hyperemia is mainly mediated by local release of ischemia-induced vasodilator substances from forearm resistance vessels , although myogenic response and endothelial nitric oxide likely play a role. Further studies are needed to investigate whether nitroglycerin-mediated dilatation of the brachial artery and reactive hyperemia in the forearm are associated with increased cardiovascular risk.

Thus, arterial dysfunction related to atherosclerosis and its risk factors may

manifest as impaired conduit artery responsiveness to endothelium-dependent and endothelium-independent stimuli as well as reduced microvascular reactivity. Assessment of conduit artery and microcirculatory function using ultrasonography could be useful in refining cardiovascular risk estimates, choosing appropriate risk-reduction therapy, and assessing the effect of therapeutic interventions. However, brachial vasoreactivity testing has significant test-to-test variability and requires a skilled ultrasonography technician. Although edgedetection software may improve precision and reproducibility and decrease dependence on operator skill, there remains a need for standardizing of measurement technique across laboratories and establishing cutoff values that differentiate normal from abnormal. Prospective studies that assess the independent predictive value of brachial vasoreactivity testing in asymptomatic subjects are awaited.

ARTERIAL STIFFNESS AND ASSESSMENT BY TONOMETRY

Arterial stiffening is a manifestation of arteriosclerosis, a process characterized by thickening and loss of elasticity of the arterial wall. Capacitance and conduit arteries are predominantly affected, and histopathological features include fractured and damaged elastin fibers and increased collagen deposition . Not only is increased arterial stiffness a marker of vascular aging, it also predicts target organ damage and cardiovascular events. Increased arterial stiffness impairs the cushioning function of the central arterial reservoir with adverse consequences for cardiac performance and organ perfusion. The resulting hemodynamic abnormalities (e.g., increased systolic blood pressure [BP] and pulse pressure and reduced diastolic BP) increase cardiovascular morbidity and mortality . Systolic hypertension increases cardiac workload and leads to myocardial hypertrophy , whereas reduced diastolic BP may compromise coronary perfusion and increase vulnerability to ischemia. Stiffening of the aorta and its major branches may reduce or eliminate the normal elastic gradient between the central and peripheral segments of the arterial tree; the resulting increase in distal transmission of pressure pulse energy is deleterious to the microvascular beds of many organs and tissues . The pulsatile stress associated with increased pulse pressure may also induce arterial remodeling, contribute to plaque formation and progression , and alter hemodynamic forces acting on the plaque surface, all of which could increase the propensity for plaque rupture .

Several indexes of arterial stiffness have been proposed. However, 2 measures have been studied extensively:

1. the velocity of arterial pulse wave transmission across an arterial segment, and

2. Analysis of the arterial waveforms to estimate augmentation of systolic pressure by peripheral wave reflection.

   Both measures may be assessed conveniently and reproducibly by using commercially available devices based on the principle of applanation tonometry [**48, 49, 50**]

AORTIC PULSE WAVE VELOCITY (aPWV).

Because blood is a noncompressible fluid, transmission of the arterial pressure wave occurs along the arterial wall and is influenced by the biomechanical properties of the arterial wall. The velocity of the pressure wave transmission (pulse wave velocity [PWV]) provides a robust estimate of arterial stiffness and is described by the Moens-Korteweg equation:

$$PWV = \sqrt{Yh/2\rho R}$$

where Y is the Young's modulus of the arterial wall, h is wall thickness, R is arterial radius at the end of diastole, and ρ is blood density.

Central arterial stiffness is most commonly estimated by measuring carotid-femoral PWV (aPWV) because the common carotid and femoral arteries are located superficially and because the distance between them spans most of the length of aorta, the arterial segment particularly prone to stiffening. Applanation tonometry provides a convenient method for measuring aPWV, although Doppler ultrasonography and magnetic resonance imaging also may be used. The latter allows accurate assessment of aortic length and provides separate estimates of PWV for the proximal and distal segments of aorta , but cost and logistics hinder its use as a screening tool. In healthy adults, aPWV generally ranges from 5 to 7 m/s.

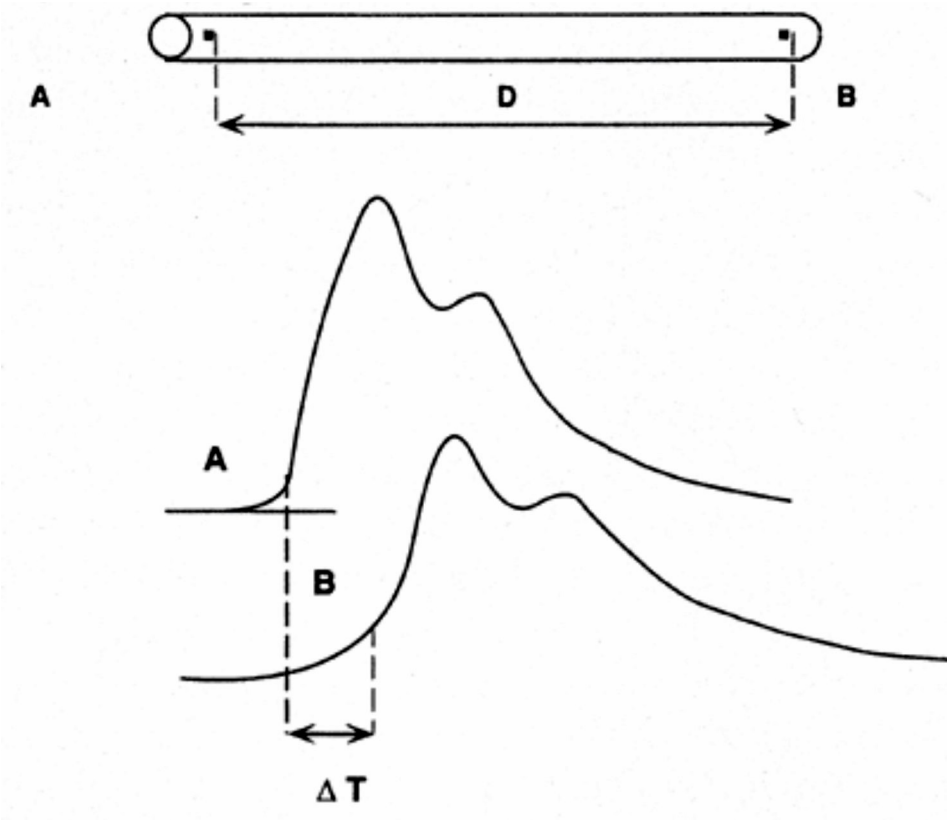


Fig.5 Principle of Measurement of Aortic pulse wave velocity

$$\textbf{(Foot to foot method) aPWV} = \frac{\mathbf{D}}{\mathbf{\Delta T}}$$

aPWV; IMPORTANT STUDIES AND OUTCOMES

Aortic PWV increases with age and with the cumulative effect of risk factors on arterial wall physiology and structure. Increased aPWV is associated with the presence and extent of atherosclerotic disease in the coronary arteries and other vascular beds . Aortic PWV is associated with cardiovascular risk factors and correlates with estimates of cardiovascular risk based on conventional risk factor algorithms[*Blacher J, Asmar R, Djane S, London GM, Safar ME. et al*] [**51**]. A growing body of evidence indicates that aPWV itself may have prognostic value. Several studies have shown an independent and significant association between aPWV and cardiovascular events in different populations , including patients with end- stage renal disease [*Blacher et al*] [**52**], hypertension [*Boutouyrie P, Tropeano AI*, *Asmar R, et al.*] [**53**] , and the elderly[*Meaume S, Benetos A, Henry OF, Rudnichi A, Safar ME et al*] [**54**] and [*Sutton-Tyrrell K, Najjar SS, Boudreau RM, et*

al] [**55**].

Two recent studies have shown that aPWV provides incremental information about cardiovascular risk in asymptomatic individuals from the general population. In a community-based study of 1,678 Danes [Willum-*Hansen T, Staessen JA, Torp-Pedersen C, et al*] [**56**], aPWV predicted incident cardiovascular events over a median follow-up period of 9.4 years. After adjustment for conventional risk factors and average 24-h ambulatory BP, a 3.4-m/s increase in aPWV was associated with a 16% to 20% increase in the risk of an adverse cardiovascular event. In the Rotterdam Study [*Mattace-Raso FU, van der Cammen TJ, Hofman A, et al.*] [**57**], aPWV was a predictor of CHD and stroke, independent of conventional risk factors, measures of atherosclerosis (carotid IMT and ankle-brachial index), and pulse pressure. The age- and gender-adjusted hazard ratio for incident cardiovascular disease in subjects with aPWV in the highest tertile compared to those with aPWV in the lowest tertile was 2.40 (95% confidence interval 1.51 to 3.83).

To summarize, aPWV is a robust measure of central arterial stiffness that may be a useful adjunct to cardiovascular risk stratification. A recent consensus document [*Laurent S, Cockcroft J, Van Bortel L, et al.*] [**48**]described aPWV as the gold-standard measure of arterial stiffness. Analogous to carotid IMT, aPWV also could be used to assess vascular age. The case for incorporating aPWV into routine clinical practice is strengthened by the evidence supporting its independent prognostic value and the availability of user-friendly devices for rapid, reliable, and reproducible measurement

PULSE WAVE ANALYSIS.

Pulse wave analysis is based on the principle that the forward-moving pressure wave generated with each cardiac pulse is partially reflected back toward the aorta at points of impedance mismatch along the arterial tree (bifurcations, branch points, arterioles, and other sites of discontinuity in arterial elasticity); this reflection increases (augments) the central aortic pressure . Applanation tonometry is a simple and reproducible method of pulse wave analysis. The technique involves partial flattening (applanation) of a superficial artery against an underlying bone using a handheld external pressure sensor; the sensor eliminates tangential pressures and

determines pressure within the artery [**58**] . A correctly obtained noninvasive pressure waveform is virtually identical to a waveform recorded by intra-arterial transducers. The radial artery pressure waveform is used to derive a central aortic pressure waveform using a mathematical transfer function that has been validated under several different conditions, although some investigators have questioned the generalizability of the transfer function . Tonometry of the carotid artery may obviate the need of a transfer function but requires a higher degree of technical expertise, particularly in obese subjects [**59**] .

Analysis of arterial pulse waveforms provides information about several hemodynamic characteristics related to arterial wave reflection. Augmentation pressure is defined as the degree of augmentation of central systolic BP by the reflected pressure wave. It is generally expressed as a fraction of the central pulse pressure (aortic augmentation index [AIx]), and it is affected by several factors, including the velocity of the reflected wave. When arteries are relatively compliant (e.g., in a young healthy adult), PWV is low; the reflected wave arrives at the ascending aorta after closure of the aortic valve and augments diastolic BP, thereby increasing coronary perfusion. With increasing arterial stiffness, the reflected wave is transmitted at a higher velocity and arrives in systole, resulting in augmentation of systolic pressure, increased cardiac workload, loss of augmentation of diastolic pressure, and decreased coronary perfusion. Whereas aPWV represents a measure of aortic stiffness, AIx reflects the overall interaction between the arterial tree and the left ventricle **[60, 61**] .

Like carotid IMT and aPWV, AIx has been proposed as a measure of vascular age, albeit only in individuals younger than 60 years . This is because AIx plateaus at or may even decrease after the age of 60 . The decrease in AIx in older individuals could be caused by a proportionally greater effect of the reflected wave in reducing flow at the aortic valve than in augmenting the systolic BP in the aorta . Further, the preferential increase in central arterial stiffness with aging and resulting changes in thenormal centrifugal gradient of arterial stiffness may result in reduced amplitude, despite earlier timing of pressure wave reflection, and decrease AIx [**62, 63**] .

PULSE WAVE ANALYSIS; IMPORTANT STUDIES AND OUTCOMES

Higher AIx is associated with increased left ventricular mass in normotensive[*Sa PS, Roman MJ, Pini R, Spitzer M, Ganau A, Devereux RB*.] [**64**] and hypertensive,[*Lekakis JP, Zakopoulos NA, Protogerou AD, et al*.] [**65**]with lower cardiorespiratory fitness in asymptomatic men , and with lower walking distance in patients with peripheral arterial disease [*Brewer LC, Chai HS, Bailey KR, Kullo* et al] [**66**]The AIx has also been associated with higher C-reactive protein levels in asymptomatic adults and with several cardiovascular risk factors . However, AIx is lower in men compared with women it is inversely related to body mass index [*van Trijp MJ, Bos WJ, Uiterwaal CS, et al*.] [**67**]and it may not be a reliable measure of arterial stiffness in diabetic patients [*Schram MT, Henry RM, van Dijk RA,* et al.] [**68**]

Cross-sectional studies have shown AIx to be associated with the presence and extent of angiographic coronary artery disease in patients with end-stage renal disease and in men _60 years of age undergoing diagnostic coronary angiography [*Weber T, Auer J, O'Rourke MF, et al*] [**69**].Whether AIx is associated with cardiovascular risk in community-based cohorts is not known, although several small studies have also shown AIx to be predictive of adverse cardiovascular events in select populations , such as patients with end-stage renal failure [*London GM, Blacher J, Pannier B, Guerin AP, Marchais SJ, SafarME*. Et al] [**70**] patients with angiographically documented coronary artery disease [*Chirinos JA, Zambrano JP, Chakko S, et* al] [**71**] and patients undergoing coronary revascularization [*Ueda H, Hayashi T, Tsumura K, Yoshimaru K, Nakayama Y, Yoshikawa J*] [**72**] A recent study did not find AIx to be predictive of cardiovascular events in elderly hypertensive women [*Dart AM, Gatzka CD, Kingwell BA, et al*.] [**73**]

Pulse wave analysis also can be used to estimate central systolic and pulse pressures from the radial artery-derived aortic pressure waveform. Because of progressive amplification of the pressure waveform from the central arteries to the peripheral arteries, systolic BP and pulse pressure from brachial artery cuff measurements may not accurately reflect the corresponding pressures in the proximal

aorta. Furthermore, with age-related changes in arterial stiffness and wave reflection, there is a disproportionate increase in central BP when compared with peripheral systolic and pulse pressures. Although sphygmomanometric measurements of brachial artery BP are well-established markers of cardiovascular risk, these are surrogates for the more important central BP to which the left ventricle is exposed. Recent reports suggest that central rather than brachial systolic BP is more relevant to left ventricular afterload and may therefore be a better marker of cardiovascular risk [*Safar ME, Blacher J, Pannier B, et al and Roman M, Kizer JR, Ali T, et al..J*.[**74,75]**

Cuff BP measurements may also underestimate the reduction in aortic BP with vasodilator therapy because these drugs specifically affect the tone of the muscular arteries without directly affecting the larger elastic arteries. The resulting changes in pressure wave reflection may produce lower central arterial systolic pressure than would be projected from the brachial pressure (measured by the cuff method), and thus, vasodilator therapy may seem to lead to cardiovascular benefits beyond BP control [*Devereux RB, Dahlof B, Gerdts E, et al. and Yusuf S, Sleight P, Pogue J, Bosch J, Davies R, Dagenais G.*].[**76, 77**] Results from the recently reported CAFE [Conduit Artery Function Evaluation] [**78**] study support this hypothesis In this study of 2,073 patients (ages 40 to 79 years) with untreated hypertension and no known cardiovascular disease, treatment with amlodipine with or without perindopril produced a significantly greater reduction in central aortic systolic and pulse pressures when compared with treatment with atenolol with or without thiazide, despite similar reductions in brachial BP. Post-hoc analysis showed that both central aortic and peripheral pulse pressures were predictive of the composite end point of cardiovascular events, revascularization procedures, and development of renal insufficiency.

In summary, measurement of arterial stiffness using tonometry is a potentially useful adjunct to cardiovascular risk stratification. Commercial tonometric devices are now available; they can be used to obtain aPWV as well as to acquire arterial pressure waveforms in an office setting. Further, analysis of the radial artery-derived aortic pressure waveform provides estimates of central aortic pressures that may be superior to cuff-derived measurements at the brachial artery as a guide to antihypertensive therapy. The information obtained from pulse wave analysis may be used to improve

characterization of hemodynamic patterns and to generate indexes of ventricular–vascular interaction that were previously possible only with invasive arterial catheterization.

WHAT IS THE RELATIONSHIP BETWEEN ARTERIAL STRUCTURE AND FUNCTION? CAROTID INTIMA-MEDIAL THICKNESS

The combined thickness of carotid artery intima and media is measured noninvasively by high-resolution ultrasonography. Although atherosclerosis is predominantly a disease of the intima, carotid IMT correlates with the degree of carotid atherosclerosis measured at autopsy , and the latter, in turn, has been found to correlate with atherosclerotic vascular disease in other arterial beds. Thus, ultrasound-derived carotid IMT is considered a surrogate for systemic atherosclerotic disease burden. In addition to IMT, carotid ultrasonography also provides information about the presence of plaque, lumen stenosis, and arterial remodeling. Increased carotid IMT is associated with cardiovascular risk factors, prevalent cardiovascular disease , coronary artery calcification on computed tomography, presence and extent of angiographically determined coronary atherosclerosis, and plaque burden on intracoronary ultrasound . Carotid IMT has been used in research settings to identify patients with subclinical atherosclerosis and as an intermediate outcome variable in epidemiologic studies and intervention trials of disease progression [**79, 80, 81**].

Several studies show that increased carotid IMT is predictive of cardiovascular events in asymptomatic individuals and recurrent events in patients with known cardiovascular disease [*Bots ML, Hoes AW, Koudstaal PJ, Hofman A, Grobbee DE. And Hodis HN, Mack WJ, LaBree L, et* al] [**82, 83**], independent of conventional risk factors. Increased IMT was associated with risk of myocardial infarction, stroke, and death in several large prospective studies with different study designs, sample characteristics, and IMT measurement protocols. In the ARIC [Atherosclerosis Risk in Communities] [**84**] study , a mean carotid IMT of _1.0 mm was associated with a hazard ratio for incident CHD events of 5.07 in women and 1.85 in men. After adjustment for risk factors, the hazard ratio associated with increased IMT was attenuated but remained statistically significant. In another study [*Salonen JT, Salonen R.*] [85], a 0.1-mm increase in common carotid artery IMT was associated

with an 11% increase in the risk of myocardial infarction, and the association remained significant after adjustment for several cardiovascular risk factors. In the Cardiovascular Health Study [**86**], 4,476 subjects were followed over a median period of 6.2 years, and the baseline carotid IMT was associated with cumulative survival free of myocardial infarction or stroke. For a 1-SD increase in baseline IMT, the age- and gender-adjusted relative risk for the combined end point increased by 35% to 44% and remained significantly elevated after adjustment for risk factors. In CAPS [**87**] [Carotid Atherosclerosis Progression Study] , 5,056 members of a German primary health care scheme were followed for a mean of 4.2 years. A higher common carotid IMT was associated with increased risk of incident myocardial infarction, stroke, and the combined end point of myocardial infarction, stroke, or death even after adjustment for conventional risk factors.

Given that carotid IMT is associated with coronary atherosclerosis and cardiovascular events, it may be useful in refining risk stratification of individual patients, especially those assigned to an intermediate-risk category. A limitation of the Framingham cardiovascular risk equations is that these are heavily influenced by patient age without taking into account the heterogeneity of atherosclerotic burden. Researchers have proposed replacing chronological age in the Framingham risk algorithms with vascular age derived from the composite carotid IMT score *[Stein JH, Fraizer MC, Aeschlimann SE, Nelson-Worel J, McBride PE, Douglas PS. andHoward G, Sharrett AR, Heiss G, et al.*] [**88, 89**]. Using such an approach, nearly one-half of the individuals assigned to the intermediate-risk category by the original Framingham CHD risk algorithm were reclassified into higher- or lower-risk categories. Computerized algorithms may be used to integrate IMT values with demographic and other relevant clinical information to determine the vascular age of the individual and recalculate the cardiovascular risk .

The reported median values of carotid IMT have varied, although a value of 1.2 mm or higher for an adult would be considered clearly abnormal . Progression of IMT in asymptomatic individuals is estimated to be less than 0.03 mm/year , but this rate is accelerated in the presence of cardiovascular risk factors . A higher progression rate is associated with increased risk of myocardial infarction and stroke; therefore, serial measurements may provide greater prognostic information than a single

measurement. Distinct from diffuse intimal-medial thickening is atherosclerotic plaque, which is typically seen as a focal thickening often with mineralization and protrusion into lumen. Some studies suggest that plaque area or volume may be a better predictor of cardiovascular events than IMT, particularly in diabetics , but the evidence is mixed . Although carotid IMT needs to be interpreted in the context of age and gender of an individual, presence of a carotid plaque is always abnormal. However, plaques develop late in atherogenesis , whereas IMT can be measured at any age and may be a better marker of systemic atherosclerosis The predictive value of carotid plaques may be greater in patients with known cardiovascular disease, whereas carotid IMT may be a better prognostic marker in asymptomatic individuals. However, in a recent study [*Touboul PJ, Labreuche J, Vicaut E, Amarenco* P] [**90**], both carotid IMT and plaque were independently predictive of stroke risk, and the predictive value of IMT was higher in the absence than in the presence of plaque. In another recent study [*Rosvall M, Janzon L, Berglund G, Engstrom G, Hedblad* B.] [**91**] of 5,163 apparently healthy middle-aged Swedish men and women, carotid IMT was associated with incident stroke, even after adjustment for presence of carotid plaque and conventional risk factors.

Ultrasonographic measurement of carotid IMT is a safe, inexpensive, and reproducible measure of atherosclerotic burden associated with prevalent and incident cardiovascular disease. Carotid IMT also can be used as a marker of efficacy of therapies intended to achieve regression of atherosclerosis . Integration of carotid IMT measurement into routine cardiovascular risk assessment may improve risk stratification of individual subjects. The American Heart Association has concluded that among asymptomatic persons more than 45 years old, measurement of carotid IMT in experienced laboratories could be considered for further clarification of CHD risk. Automated computerized edge-detection software and intravascular contrast agents may decrease variability and improve precision in IMT measurement. Development of guidelines for quality control, standardization of measurements, and establishment of thresholds for different risk categories will help optimize the use of carotid IMT in clinical practice.

CIRCULATING MARKERS OF ENDOTHELIAL FUNCTION

A broader appreciation of the numerous functions of the endothelium can be obtained by study of the levels of molecules of endothelial origin in circulating blood. These include direct products of endothelial cells that change when the endothelium is activated, such as measures of NO biology, inflammatory cytokines, adhesion molecules, regulators of thrombosis, as well as markers of endothelial damage and repair. Many of these circulating markers are difficult and expensive to measure, and currently are only used in the clinical research setting. In this context, these measures can provide important information regarding mechanisms and severity of endothelial dysfunction in populations, and complement physiological tests of endothelial vascular control. As a result of biological and assay availability and variability, these factors currently have only a very limited role in the assessment of individual patients Currently the following molecules are being studied

1. Circulating levels of nitrites and nitrosylated proteins
2. Asymmetric dimethylarginine
3. E-selectin
4. Vascular cell adhesion molecule 1
5. Intercellular adhesion molecule 1
6. P-selectin
7. Tissue plasminogen activator and its endogenous inhibitor,plasminogen activation inhibitor-1
8. Von Willebrand factor
9. Balance between circulating endothelial progenitor cells and mature endothelial cells

COMPARATIVE VALUE OF NON INVASIVE TESTING MODALITIES

The arterial tests discussed in this review assess different aspects of arterial function and structure and are not strongly correlated with each other; for example, brachial artery FMD correlates weakly with carotid IMT and with measures of arterial stiffness . Previous studies have shown that carotid plaque burden and brachial artery FMD independently and additively predict occurrence of cardiovascular events and that aPWV may be a predictor of CHD and stroke, independent of carotid IMT. Thus, different arterial tests may provide complementary information about arterial structure and function, and a combination of the tests might be superior to any single test used alone. Further, because some arterial changes (e.g., impaired FMD) may be discernible earlier than others (e.g., increase in carotid IMT or aPWV), an absence of abnormality in one testing domain would not rule out an abnormality in other domains.

The relative utility of various modalities and tests as adjuncts to cardiovascular risk assessment depends on several factors. Tonometry has an advantage over ultrasonography in that it can be performed after minimal training using a relatively low-cost portable device, whereas ultrasound assessment of IMT and FMD require relatively expensive equipment and considerable operator skill. Carotid IMT and aPWV are predictive of cardiovascular events in community-based cohorts, independent of conventional risk factors, whereas brachial artery FMD and AIx have been shown to be associated with cardiovascular events in select groups of patients.

MATERIALS AND METHODS

The study was conducted in the Cardiology Department of Madras Medical College ,Government General Hospital ,Chennai-03.

STUDY DESIGN

Patients attending the Cardiology out patient Department for evaluation of chest pain or dyspnoea or referred by Medicine department for evaluation of cardiovascular risk status were initially screened. Those patients in the age group of 30 to 55 years, with no previous cardiovascular disease but with multiple conventional risk factors categorized by Framingam risk score as low or intermediate risk were included and taken up for treadmill ecg stress test. Patients with inconclusive ecg stress test were included in the study. Patients with established cardiovascular diseases like previous myocardial infarction, acute coronary syndromes, stable angina, stroke, peripheral vascular disease, and renal dysfunction, elevated ESR or WBC, active systemic illness, morbid obesity were excluded from the study. All patients gave written, informed consent, and the study was approved by the Human Ethics Committee of Madras Medical College Government General Hospital ,Chennai-03.

A full clinical history and examination was done by a Cardiologist. Baseline demographic data, cardiovascular risk factors, and cardiovascular medications were documented, and a 12-lead electrocardiogram and Echocardiogram was reviewed. Routine biochemical analysis was done. Patients then underwent Flow Mediated Dilatation of Brachial Artery, Aortic pulse wave velocity, Carotid intima medial thickness and then either coronary angiogram or 64 slice CT Angiogram.

CLINICAL EVALUATION

The presence of CAD was defined as a history of MI, coronary revascularization, or typical chest pain with a positive stress ecg. Diabetes mellitus was defined by the use of insulin injections or oral hypoglycemic agents.

Hypertension was defined as an average systolic blood pressure more than140 mm Hg or diastolic blood pressure more than 90 mm Hg on three separate occasions or by the use of antihypertensive medications . Hypercholesterolemia was defined as a fasting total cholesterol level of 200 mg/dl or by the use of a statin. Smoking status was defined as current smoker or reformed/nonsmoker. Significant renal impairment was defined as chronic renal impairment, with a calculated glomerular filtration rate of less than 60 ml/min/1.73 m2 .Ten year coronary heart disease event risk in patients was calculated using the Framingham risk score , using the variables of gender, age, total cholesterol, high-density lipoprotein (HDL) cholesterol, systolic blood pressure, and smoking status. Obesity is defined as BMI >25 kg/m2 and upper limit of normal waist circumference is 80 cm for women and 90 cm for men as defined for asian population.

BIOCHEMICAL ANALYSIS.

Blood for biochemical analysis was obtained from fasting venous samples. Total cholesterol, HDL cholesterol, and triglycerides were determined by standard enzymatic methods. High-density lipoprotein was measured as a homogeneous assay in liquid phase. The Friedewald equation was used to calculate low-density lipoprotein.

FLOW MEDIATED DILATATION.

Endothelium-dependent FMD of the brachial artery was examined noninvasively using an established method of high-resolution vascular ultrasound with an upper arm cuff position. Patients were in fasting state and no intake of caffeinated drinks or smoking 4 hours allowed before the study. All vasoactive medications were stopped 48 hours before study. Brachial-artery two-dimensional and pulsed Doppler flow velocity signals were obtained above the antecubital crease with a 7.5-MHz linear array transducer using a vascular ultrasound system. Hyperemia was induced by inflating a blood pressure cuff on the proximal portion of the arm to occlude arterial flow (≥200 mm Hg) for 5 min and then rapidly deflating the cuff. The hyperemic flow is measured by pulsed Doppler within 15 seconds of cuff deflation and the maximum diameter of brachial artery is measured at 60 seconds of deflation

After a 10-min rest period to allow restoration of baseline conditions, nonendothelium-dependent brachial-artery dilation was assessed by obtaining two-dimensional images before and 3 min after administration of sublingual nitroglycerin (NTG) (0.4 mg). An investigator blinded to image sequence and clinical information performed off-line analysis of digitized end-diastolic images .

AORTIC PULSE WAVE VELOCITY.

aPWV can be measured noninvasively, and the technique has been found to be highly reproducible, with replicate testing yielding an intraclass correlation _0.80. aPWV was measured using commercial available arterial tonometer and pulse wave analysis software system by recording pulse wave signals from the right carotid and right femoral arteries with transcutaneous tonometer probe with ECG synchronization . Digitized data were recorded by custom programming for subsequent analysis. A minimum of 10 beats were averaged for each simultaneous recording site using the QRS for synchronization. Three separate runs were recorded for each participant, and all usable runs were averaged. The distance between the carotid and femoral sampling sites was measured above the surface of the body with a metal tape measure. This was done to avoid overestimation of the distance portion of the aPWV equation. The time differentials between the onset of pulse wave at carotid and femoral (defined as foot of the pressure tracing at each site) sites were divided by the associated distance to produce wave velocity.

CAROTID INTIMA MEDIAL THICKNESS.

The patients also underwent measurement ofcarotid intimal medial thickness by an already described protocol.

CORONARY ANGIOGRAM/64 SLICE CT ANGIOGRAM

The patients were subjected to either invasive coronary angiogram or 64 slice ct angiogram as “gold standard” for documenting coronary artery disease.

STATISTICAL ANALYSIS

All data are expressed as mean value +/-SD or frequency (%), unless otherwise stated. The baseline clinical characteristics of the groups were compared using the two-tailed independent *t* test for continuous variables and the chi-square or Fisher exact test for non-continuous variables, as appropriate. The outcomes were compared using the log-rank test. Independent predictors of events were calculated using Cox proportional hazards regression. The following variables were used first in a univariate model: age, gender, hypertension, hyperlipidemia, total, low-density lipoprotein, and HDL cholesterol, triglycerides, Framingam risk score. diabetes mellitus, current smoking, FMD post cuff and post NTG, aPWV,CIMT. Factors with a value of p less than 0.20 were then entered into a forward stepwise multivariate Cox proportional hazards analysis. Statistical significance was assumed at p less than 0.05. All statistical analyses were performed using SPSS for Windows 11.0 .

RESULTS

CLINICAL CHARACTERISTICS

The study population had 74 patients with 52 men and 22 women. The mean age was 43.96+/- 2.9.Diabetics constituted 35.14% and systemic hypertension was present in 27.4% of study population.There were 17 current smokers [22.97%]. The mean BMI was 26.13, mean waist circumference was 89.15, mean systolic blood pressure was 130.74, mean diastolic blood pressure was 78.93, mean fasting blood sugar was 102.61, mean total cholesterol was 210.20, mean low density lipoprotein was 120.54, mean high density lipoprotein was 45.39, mean triglycerides was 127.89, mean Framingham risk score was 8.76. There were 8 patients with FRS less than 5, 48 patients with FRS between 6 and 10, and 18 patients with FRS more than 10.

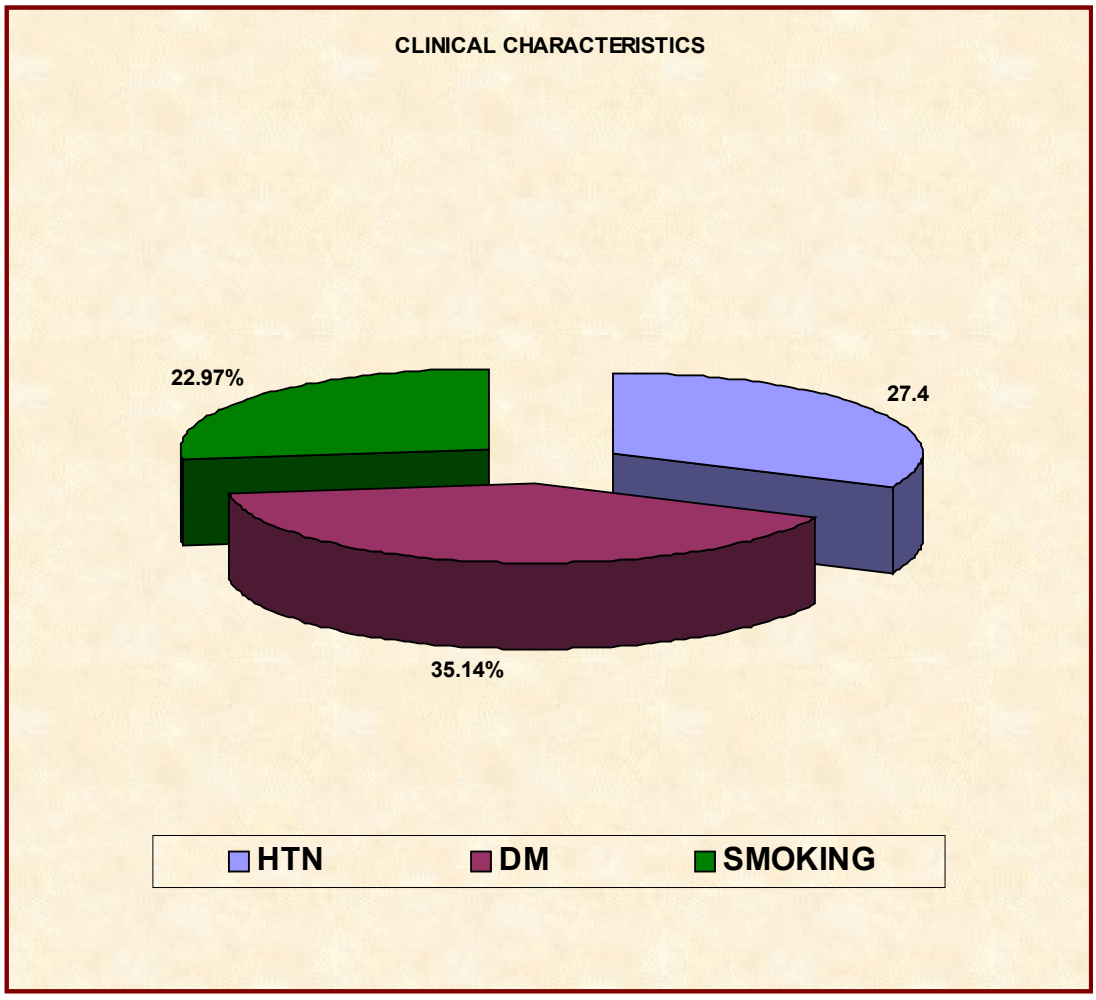


Fig.10A

IMAGING RESULTS AND OUTCOMES

The mean post cuff FMD was 8.66% +/- 2.64, mean post NTG FMD was 13.98% +/-3.07, mean baseline diameter of brachial artery was 3.68 +/-0.49, mean aortic pulse wave velocity was 11.56 m/s +/-1.64and mean carotid intima medial thickness was 0.65mm +/-0.10.

Out of 74 patients 31 patients had documented coronary artery disease as defined as atleast 50% stenosis in one /more coronary arteries by coronary angiogram/ ct angiogram.

Flow mediated dilatation was initially treated as continuous variable and then as discrete variable.Previous studies have shown that post cuff FMD less than 8% and post NTG FMD less than 15% represent abnormal FMD after taking into consideration the wide biologic variability of FMD.In our study both post cuff [representing nitric oxide mediated endothelium dependent dilatation]and post NTG [representing endothelium independent ,direct smooth muscle stimulation] concurred with each other.

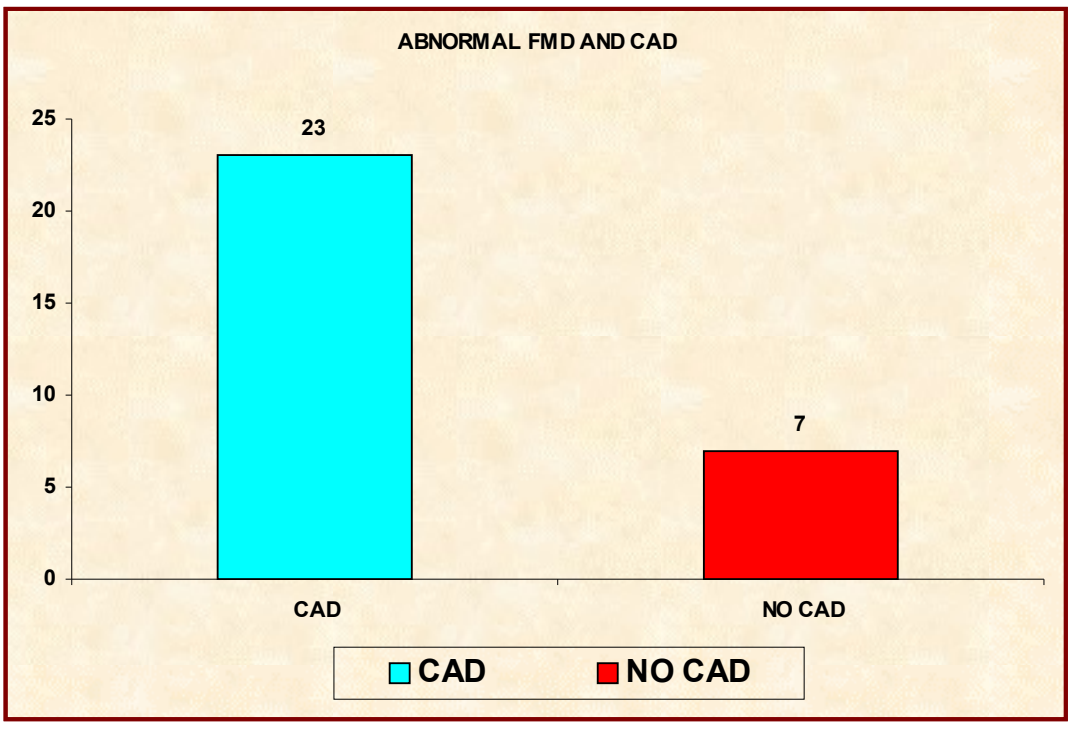


Fig.10B

Abnormal FMD was present in 30 patients in our study population out of which 21 were men and 9 were women.Among this 30 patients with abnormal FMD 23 patients had documented CAD[17 men and 6 women] . Remaining 7 patients [4 men and 3 women]had no CAD.Among 23 patients with abnormal FMD and documented CAD, carotid intima medial thickness was increased in 14 patients only implying functional changes occur before structural changes atleast in the intermediate risk patient population.Aortic pulse wave velocity was increased in 16 patients in the abnormal FMD plus documented CAD group.Taken together FMD has a positive predictive accuracy of 74.2% whereas carotid intima medial thickness has 51.6% and aortic pulse wave velocity has 58.0% accuracy.

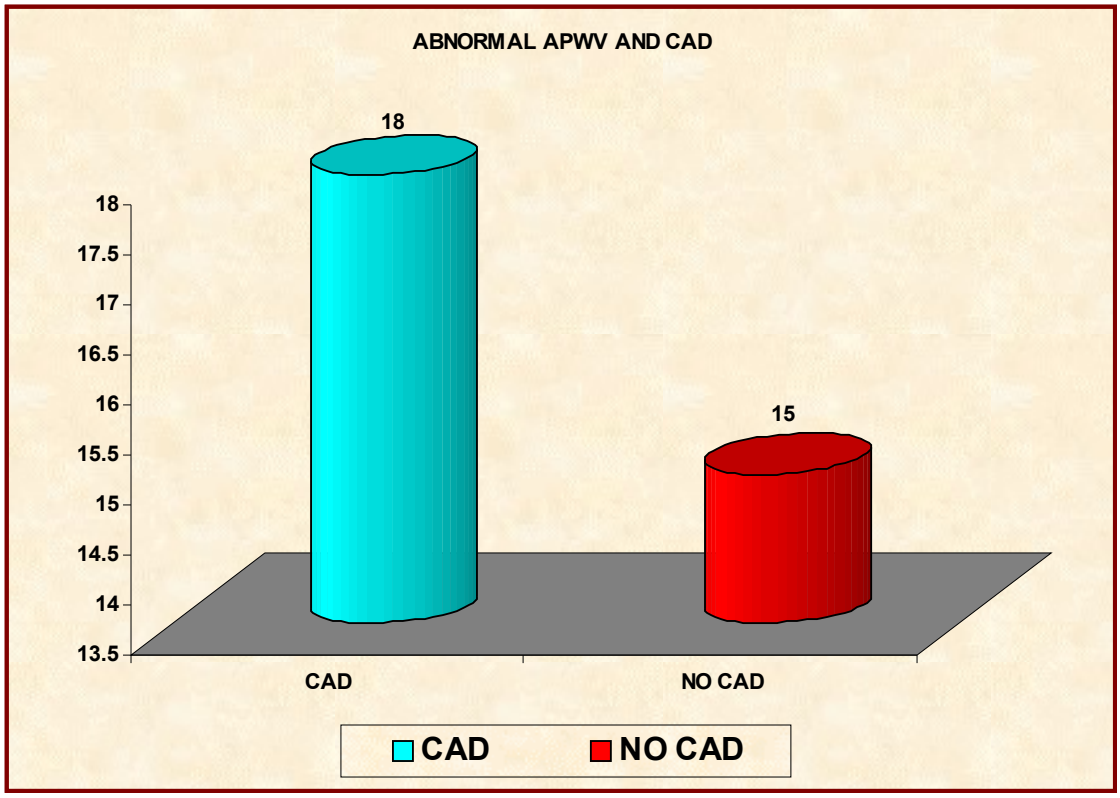


Fig.10C

Normal FMD was present in 44 patients[31 men and 13 women] in our study population of 74 patients.Out of this 36 patients[25 men and 11 women] had no CAD and 8 patients[6 men and 2 women] had documented CAD. Among 36 patients [normal FMD plus no CAD group] carotid intima medial thickness was increased in 4 patients and aortic pulse wave velocity was increased in 10 patients. In the 8 patients [normal FMD and documented CAD group] carotid intima medial thickness was increased in 2 patients and aortic pulse wave velocity was increased in 2 patients. Overall this translates into a negative predictive accuracy of 81.2% for FMD whereas carotid intima medial thickness has 72.2% and aortic pulse wave velocity has 68.3% negative predictive accuracy.

When all the three non invasive imaging modalities were combined together the positive predictive accuracy improved only marginally compared to FMD alone, meaning using FMD alone will help in better utilization of resources cutting both cost and time.

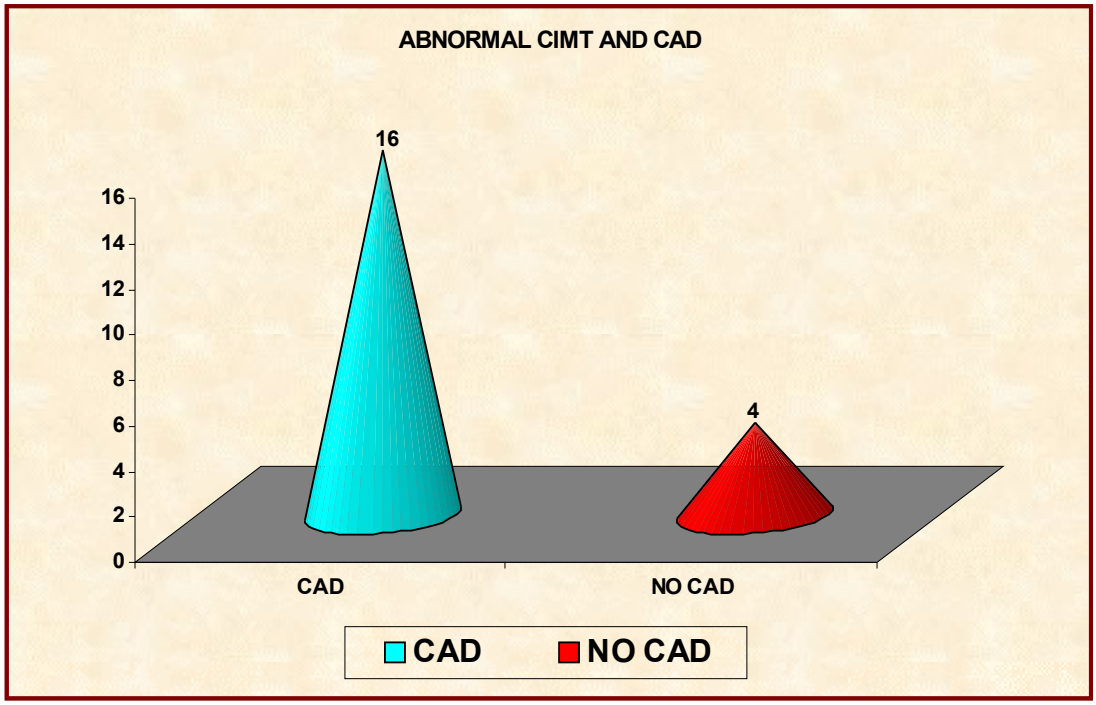


Fig.10D

A univariate analysis of clinical characteristics of patients for prediction of

CAD and correlation with abnormal FMD did not reveal any significant correlation. Multivariate predictors of CAD in patients with CAD with more than one univariate predictor revealed that in patients with CAD, independent predictors included hyperlipidemia, smoking and abnormal FMD. On comparison with angiographic disease only FMD was statistically significant with a p value less than 0.01 whereas aortic pulse wave velocity with p value of 0.57 and carotid intima medial thickness with a p value of 0.62 were statistically insignificant.

TABLE – 1

CLINICAL CHARACTERISTICS

Variable	Mean	SD
Age	43.96	2.90
BMI	26.13	2.41
Waist Circumference	89.15	7.08
Systolic BP	130.74	11.78
Diastolic BP	78.93	14.81
FBS	102.61	13.05
TC	210.20	36.17
LDL	120.54	15.99
HDL	45.39	4.87
TGL	127.89	23.29
FRS	8.76	2.84

TABLE – 2

CLINICAL CHARACTERISTICS

Variable	No	Percentage
MEN	52	70.27
WOMEN	22	29.73
HTN	20	27.4
DM	26	35.14
SMOKING	17	22.97

TABLE – 3

IMAGING

VARIABLE	MEAN	SD
FMD Post-Cuff	8.66	2.64
FMD Post-NTG	13.98	3.07
Baseline Diameter	3.68	0.49
a PWV	11.56	1.64
CIMT	0.65	0.10

TABLE – 4

APWV

APWV	CAD Group	Normal Group	PPV	NPV	Angiographic Correlation 'P'
Normal APWV (41)	13	28	58.0 %	68.3 %	0.57
Abnormal APWV (33)	18	15			

TABLE – 5

FMD

FMD	CAD Group	Normal Group	PPV	NPV	Angiographic Correlation 'P'
Normal FMD (44)	8	36	74.2 %	81.2 %	< 0.01
Abnormal FMD (30)	23	7			

TABLE – 6

CIMT

CIMT	CAD Group	Normal Group	PPV	NPV	Angiographic Correlation 'P'
Normal CIMT (54)	15	39	51.6 %	72.2 %	0.62
Abnormal CIMT (20)	16	4			

TABLE – 7

FMD Vs Angiographic CAD

	CAD Group		Normal Group	
	Mean	SD	Mean	SD
FMD - Post-Cuff	7.18	2.27	9.73	2.36
FMD - Post-NTG	12.44	2.85	15.09	2.75

DISCUSSION

Many recent studies have shown that endothelial function may serve as an integrating index of risk factor burden and genetic susceptibility, and that endothelial

dysfunction will prove to be a valuable independent marker of cardiovascular disease

According to the response-to-injury model of atherosclerosis, various factors can cause dysfunctional alterations in the overlying endothelium. This injury may then predispose arteries to the development of atherosclerosis, eg, by increasing the adhesiveness of the endothelium to leukocytes, by changing its permeability, and by inducing endothelial expression of vasoactive molecules favoring atherogenesis. This model thus predicts that arterial endothelial damage or activation is required before risk factors can induce atherosclerotic changes in the arterial wall

Several risk factors related to atherosclerosis have also been linked to endothelial dysfunction, presumably because of increased oxidative stress. However, recent studies have also shown that individuals with normal endothelial functions and various stages of endothelial dysfunction do not necessarily differ in their risk factor profiles. Al Suwaidi et al observed among 157 patients with mildly diseased coronary arteries that the proportion of hyperlipidemic, hypertensive, or smoking subjects did not differ across the groups with or without endothelial dysfunction. Similarly, Gokce et al found no difference in the proportion of these 3 main risk factors for CHD among 187 patients undergoing vascular surgery between subjects with normal endothelial function and mild or severe dysfunction. **[92, 93**]

In our study we foccused on a narrow but an important spectrum of patient population.Many middle aged men and women presenting with chest pain or dyspnoea and positive for multiple coronary artery disease conventional risk factors after initial clinical , biochemical , resting ecg and echo studies are placed in low or intermediate risk category and subjected to stress ecg. The subjects with positive and negative stress ecg are excluded from the study and managed appropriately. For those subjects with inconclusive stress ecg the next options are dobutamine echo, stress thalium scan, 64 slice ct angiogram or conventional angiogram. We hypothesized that in this cohort of subjects non invasive assessment of endothelial function by brachial artery flow mediated dilatation and aortic pulse wave velocity will further refine the risk and save unnecessary invasive angiograms and will save both cost and time.

In our study population brachial FMD demonstrated a good correlation for the presence of coronary artery disease when conventional angiogram or 64 slice ct angiogram is used as gold standard. Brachial FMD had a positive predictive value of 74.2% and negative predictive value of 81.2% for detection of coronary artery disease. Aortic pulse wave velocity taken alone showed a trend towards a better prediction but was not significant. It had a positive predictive accuracy of 58.0% and negative predictive accuracy of 68.3%. Carotid intima medial thickness a measure of arterial structural alteration to our surprise showed a weak correlation with both brachial FMD and presence of disease.

Both brachial FMD and Aortic pulse wave velocity are continuous variables in a population. Since we were also focusing on the practical utility of both these modalities, after careful and meticulous analysis of previous general population and disease based studies we assigned a cut off value and treated them as diecrete variable.For brachial FMD post cuff value of less than 8% and post NTG value less than 15% represented either lowest tertiles or 75 th percentile in studies. For Aortic pulse wave velocity assigning a discrete value was very difficult and for men a value more than 12.5 m/s and for women a value more than 11.5 m/s is taken as abnormal after referencing the highest and lowest tertiles of population and disease based studies. For carotid intima medial thickness we used well validated age and sex adjusted nomogram.

Previous studies by Raitakari OT et al, Davis PH et al and Li S et al [**94**] have shown that childhood risk factors predict increased carotid IMT in adulthood. This association seems to be independent of current risk factors, ie, suggesting that exposure to risk factors in childhood may induce permanent effects on arteries that contribute to the development of future atherosclerosis. Their prospective data suggested that enhanced vascular endothelium function in adulthood may offer some protection for arteries against the development of atherosclerosis in response to early-life exposure to risk factors.

In our study univariate logistic regression analysis did not show any significant association with individual risk factors and brachial FMD .Systolic blood pressure and age directly correlated with Aortic pulse wave velocity. Age and

hyperlipidemia had a direct association with carotid intima medial thickness. Multivariate predictors of CAD in patients with CAD with more than one univariate predictor revealed that in patients with CAD, independent predictors included hyperlipidemia,smoking and abnormal FMD. Significantly there was no correlation between multiplicity of conventional risk factors and Framingham risk model and abnormal FMD in this study population . This fact is in line with concept that FMD could represent a unique measure of vascular health, which may be influenced significantly by parameters currently not measured. Such parameters may overwhelm the influences of traditional risk factors, especially in a population with a low prevalence of traditional risk factors.

Our data did show a positive association between brachial FMD and aortic pulse wave velocity but it was not significant.The association between brachial FMD and carotid intima medial thickness was also not significant. Together these observations suggest that

1. The endothelial status may not be determined solely by the individual risk factor burden,

2. The status of brachial endothelial function would modify the association between risk factors and atherosclerosis,

3. Brachial flow mediated dilatation represents global endothelial function and has an independent and incremental information over and above the current risk assessment.

A Proposed Algorithm Incorporating Arterial Testing in the CAD Management

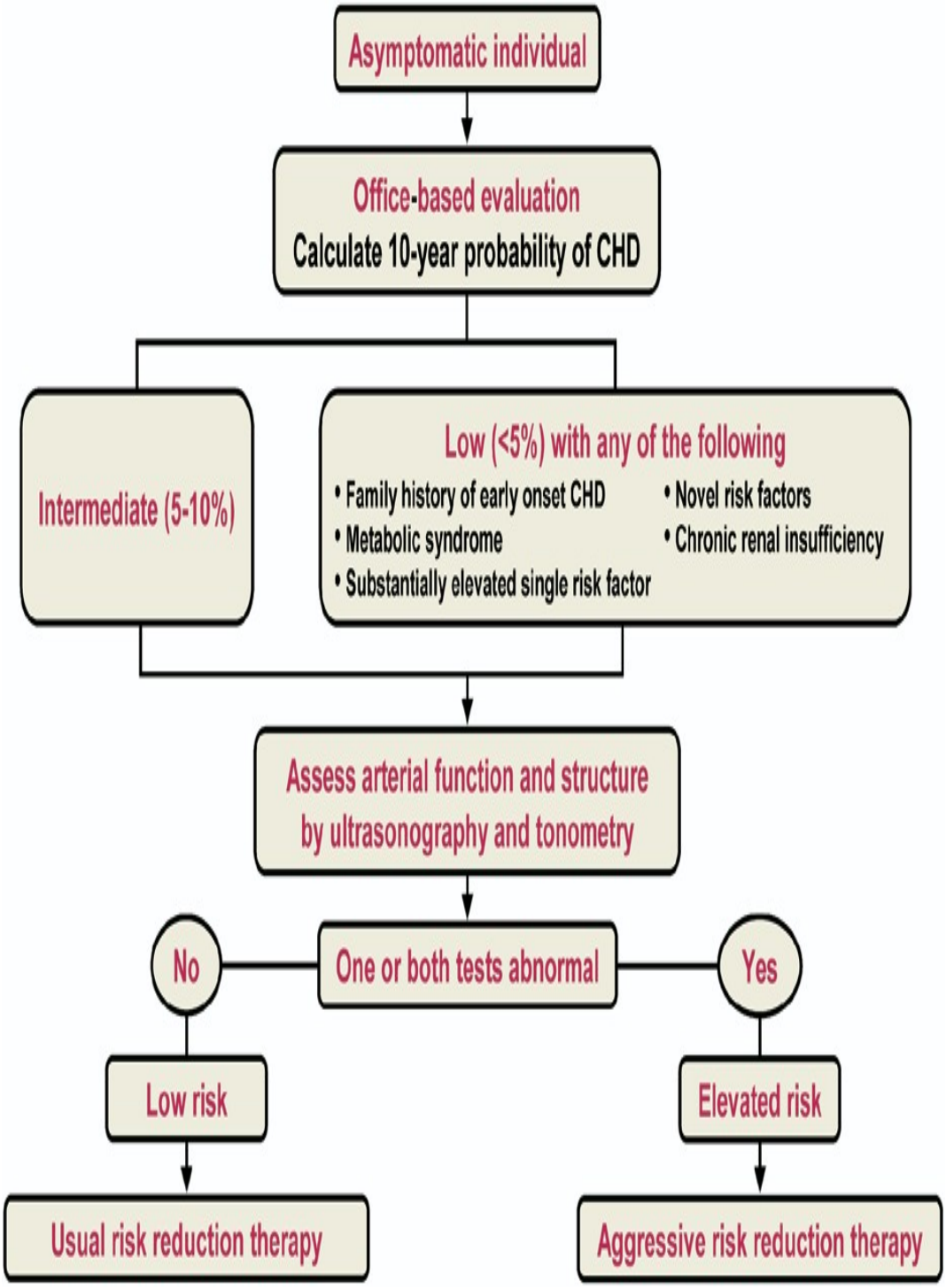


Fig.11 Arterial ultrasonography and tonometry may be useful in refining estimates of coronary heart disease (CHD) risk in intermediate-risk and select low-risk individuals.

CONCLUSION

Brachial artery flow mediated dilatation represents global endothelial function and is significantly associated with the presence of angiographically documented atherosclerosis in a cohort of middle aged south indian population with multiple coronary risk factors.

Aortic pulse wave velocity is weakly associated with both brachial flow mediated dilatation and angiographic disease.

Both brachial flow mediated dilatation and aortic pulse wave velocity correlated weakly with carotid intima media thickness

Thus, brachial flow mediated dilatation when done with meticulous attention to details is safe,inexpensive,rapid,reliable and reproducible and integration of same in routine clinical practice will provide incremental and additive information over conventional risk assessment strategy and better patient care.

Development of guidelines for quality control, standardization of measurements, and establishment of thresholds for different risk categories will help optimize the use of brachial flow mediated dilatation in clinical practice.

LIMITATIONS OF STUDY

The study was done in a narrow spectrum of middle aged population and therefore not generalizable.

The cutt off values used for both flow mediated dilatation and aortic pulse wave velocity represented either extremes of tertiles or percentiles of previous population based studies ,but consensus is still lacking.

Coronary angiogram or 64 slice CT angiogram was used as gold standard for coronary artery disease

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GLOSSARY AND ACRONYMS

FMD ;Flow Mediated Dilatation

aPWV ; aortic pulse wave velocity

A Ix ;Augmentation index

CIMT ;carotid intima medial thickness

NO ;Nitric Oxide

CAD ;coronary artery disease

CAG ;coronary angiogram

PW ;pulse wave Doppler

CW ;continuous wave Doppler

NOS ;Nitric Oxide Synthase

ROS ;Reactive Oxygen Species

EPC ;endothelial progenitor cells

EC ;endothelial cells

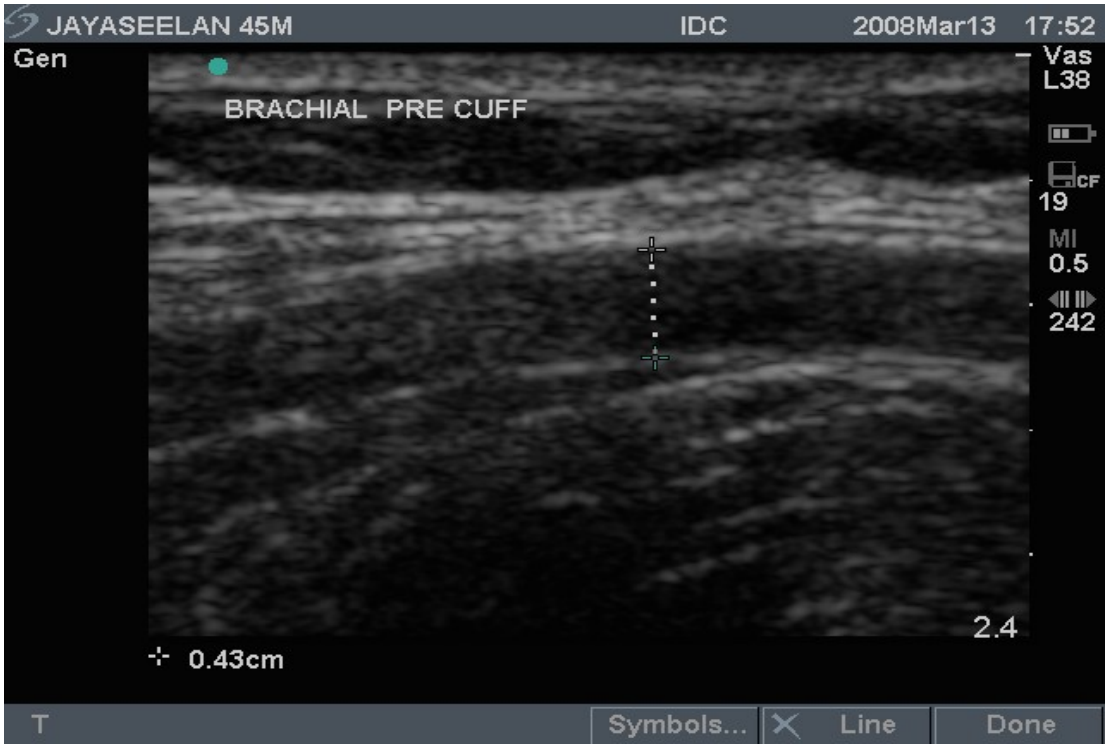


Fig.6A Patient Mr.J. Pre Cuff Baseline Diameter of Brachial Artery

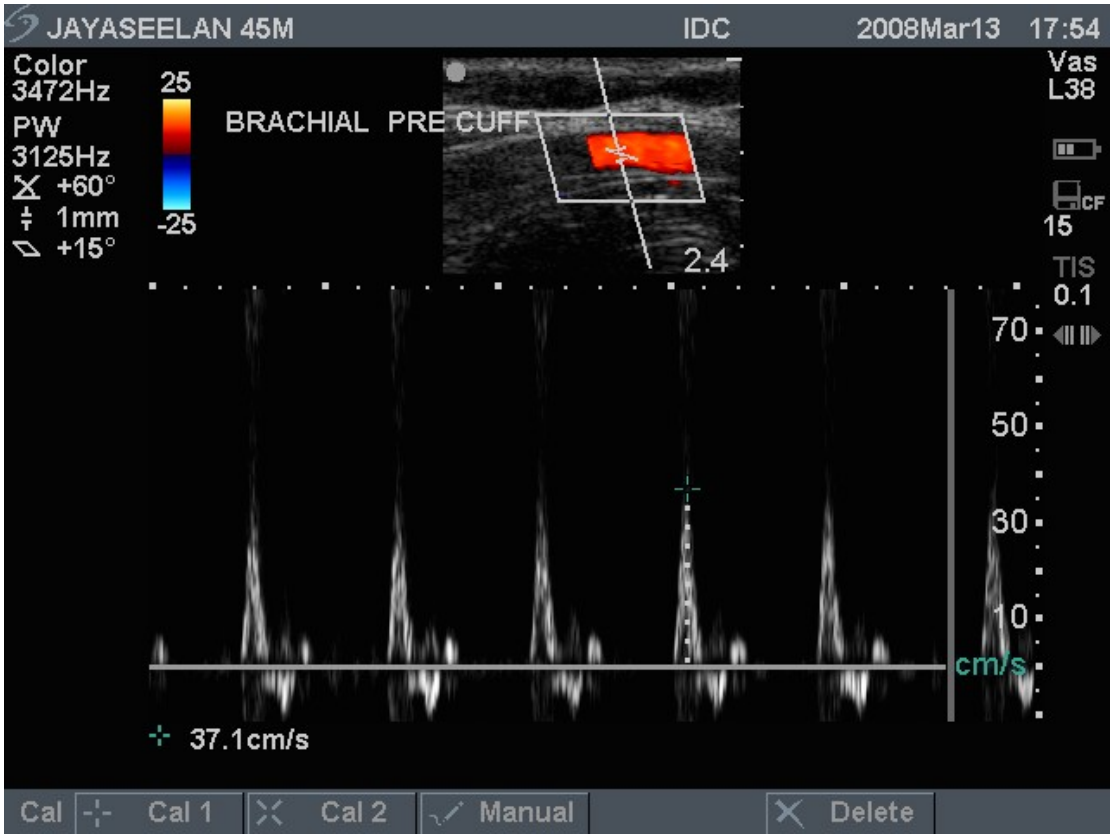


Fig.6B Patient Mr.J. Pre Cuff Baseline Brachial Artery Flow

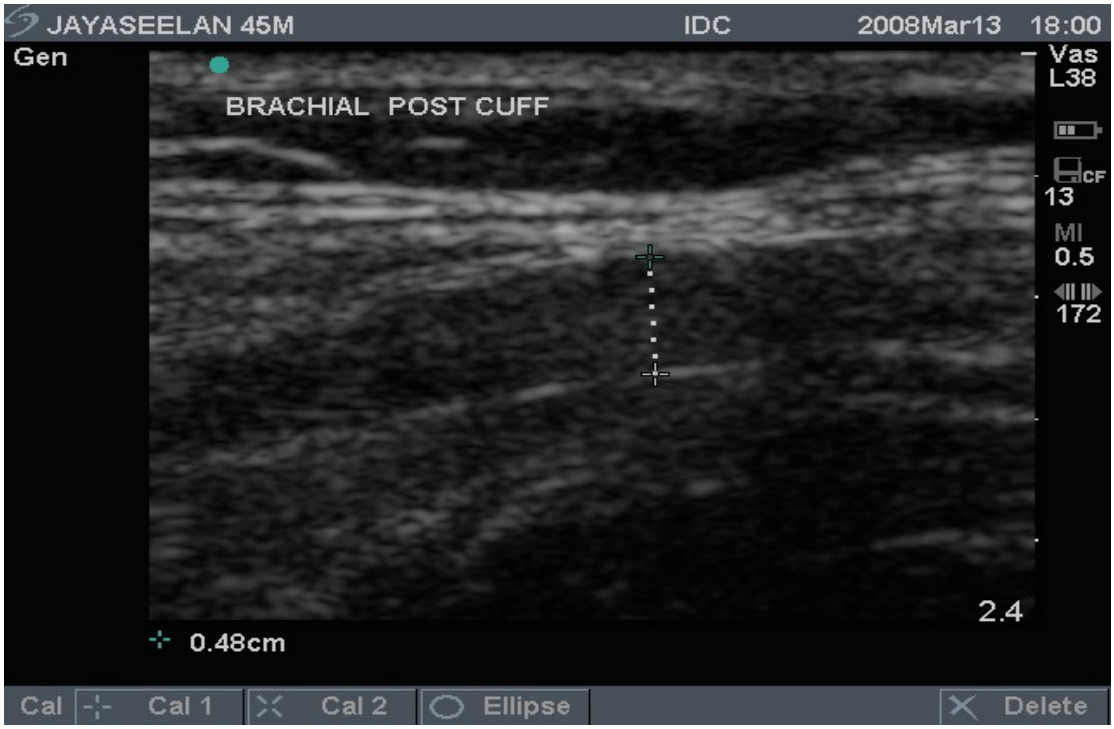


Fig.6C Patient Mr.J. Post Cuff Diameter of Brachial Artery

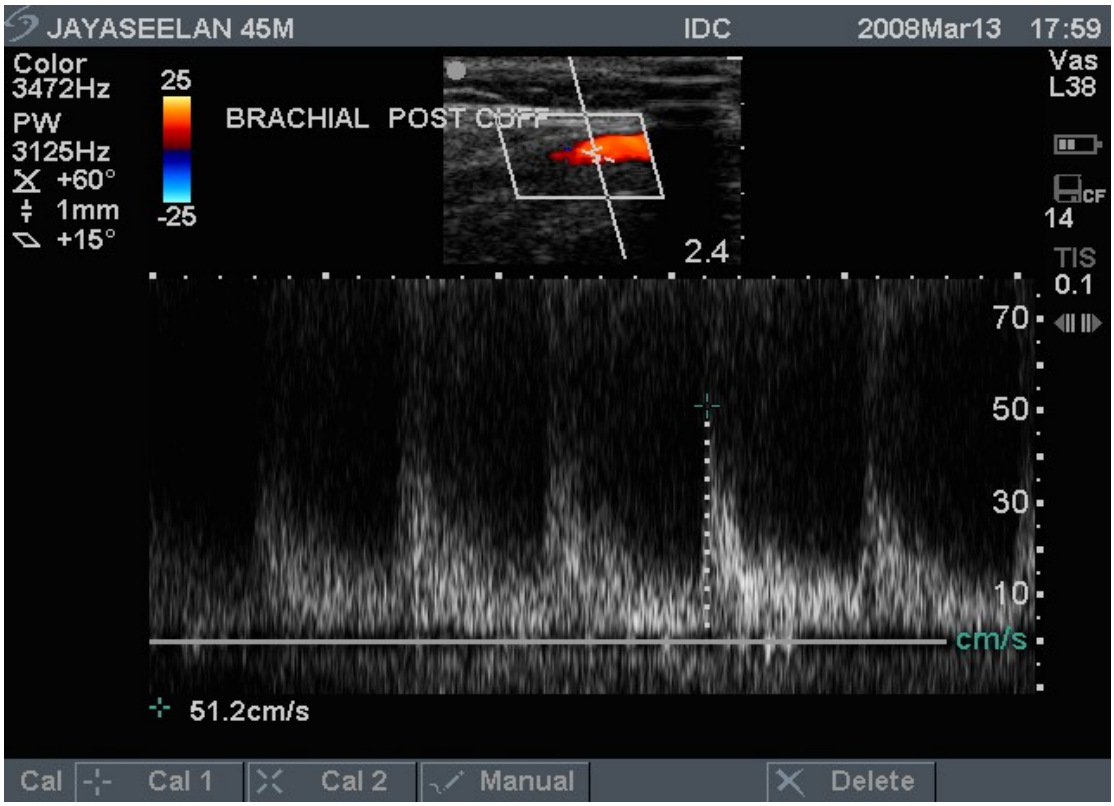


Fig.6D Patient Mr.J. Post Cuff Brachial Artery Flow

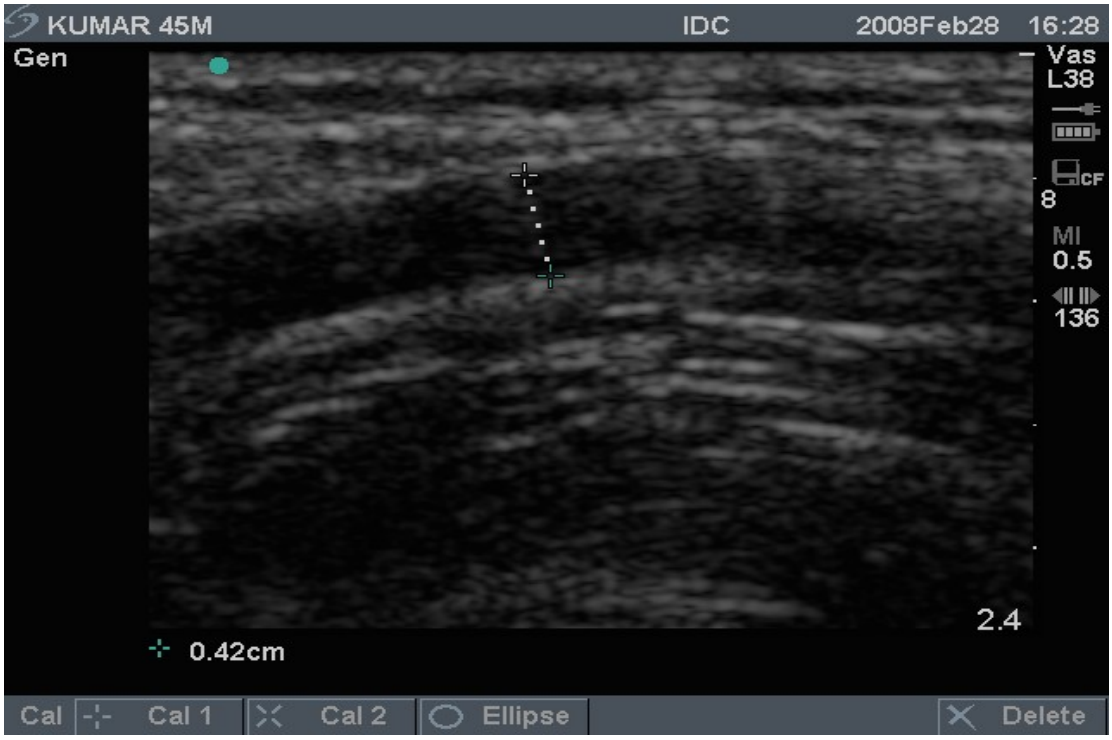


Fig.7A Patient Mr.K. Pre Cuff Baseline Diameter of Brachial Artery

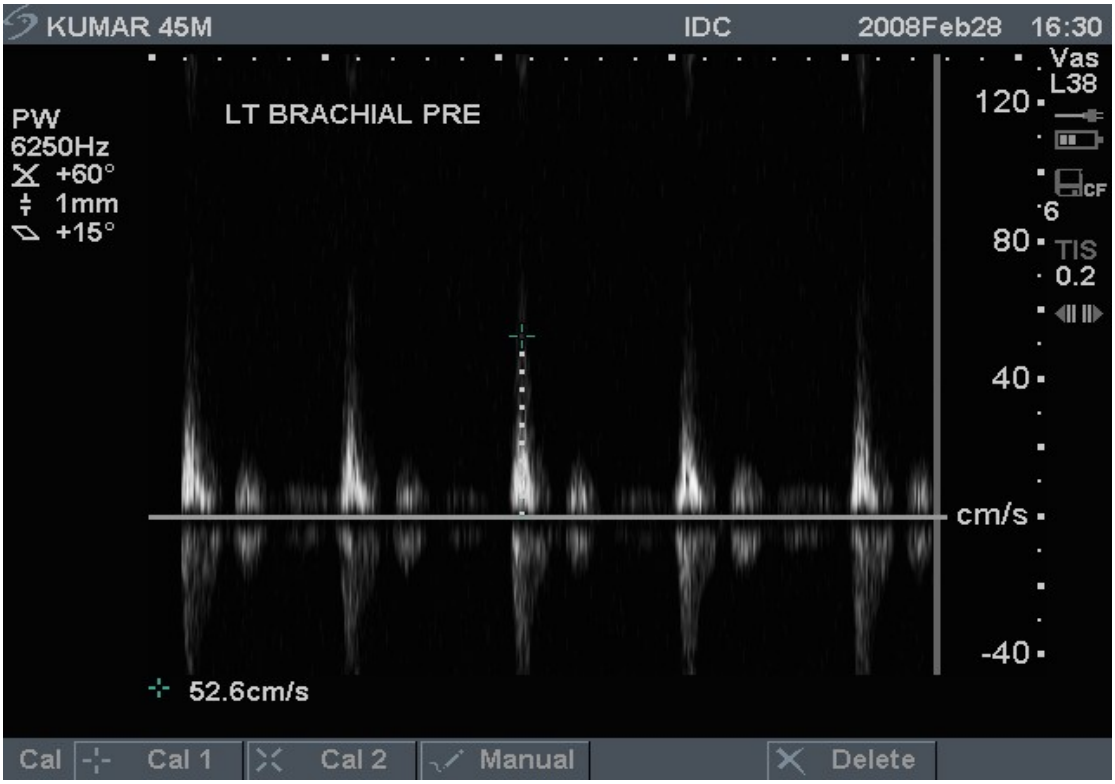


Fig.7B Patient Mr.K. Pre Cuff Baseline Brachial Artery Flow

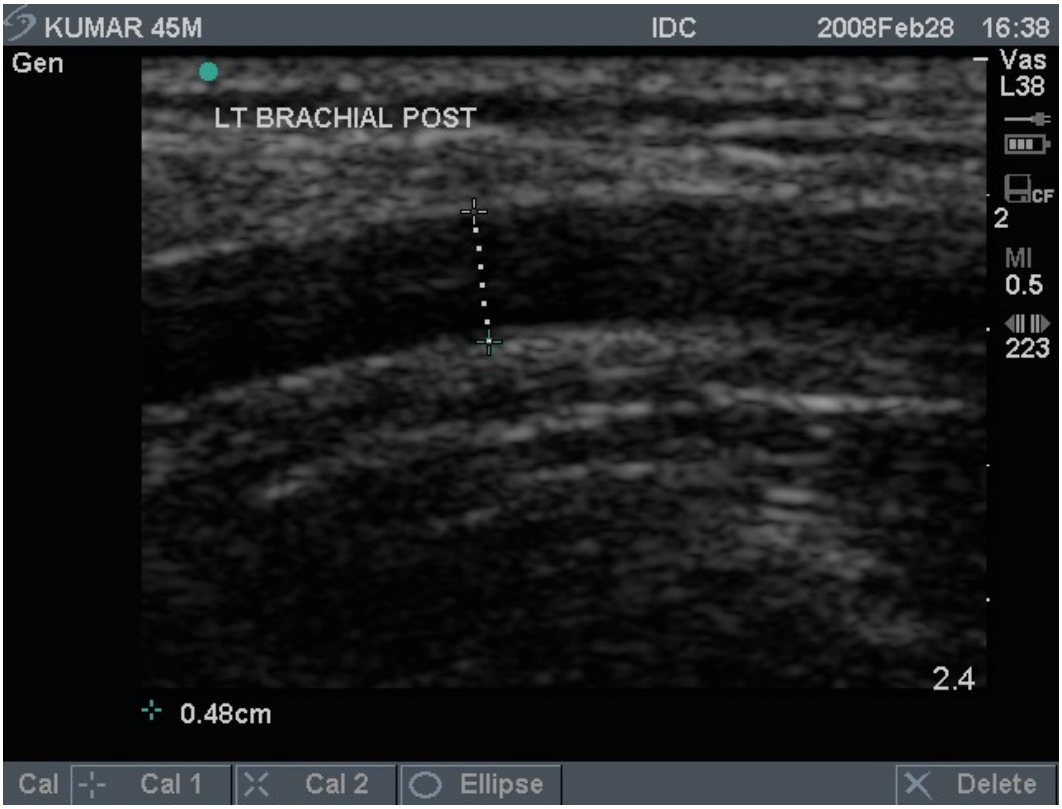


Fig.7C Patient Mr.K. Post Cuff Brachial Artery Diameter

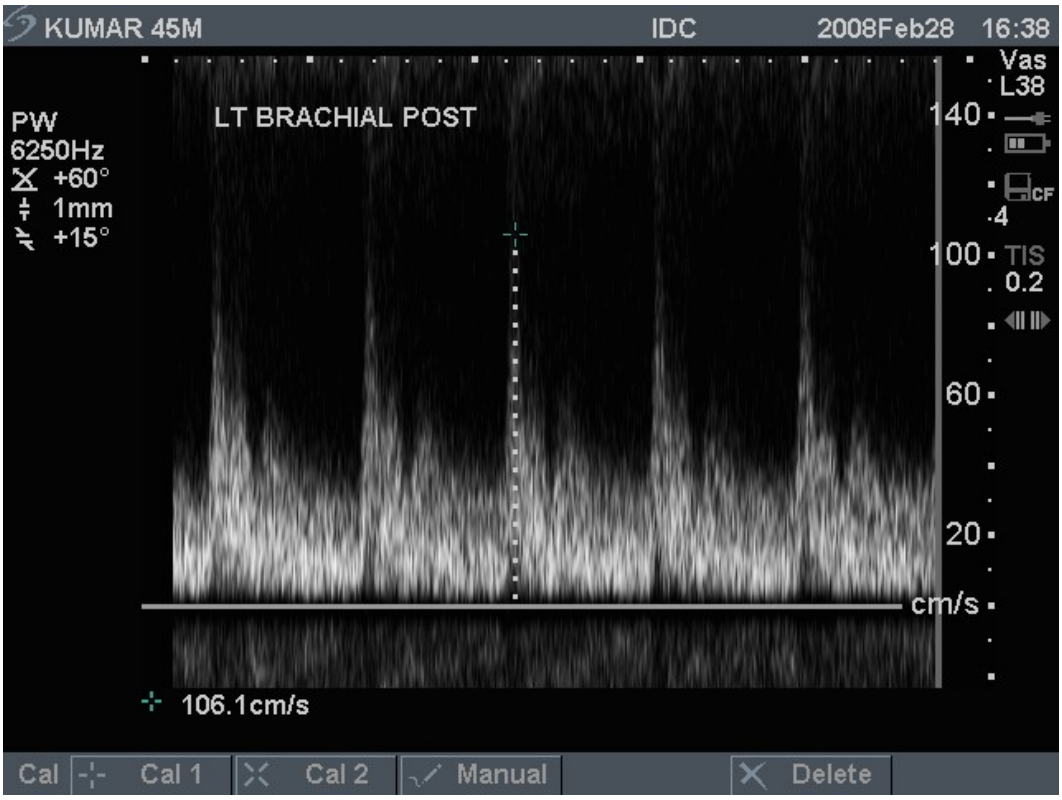


Fig.7D Patient Mr.K. Post Cuff Brachial Artery Flow

Fig.8A Patient Mr.M. Pre Cuff Baseline Brachial Artery Diameter

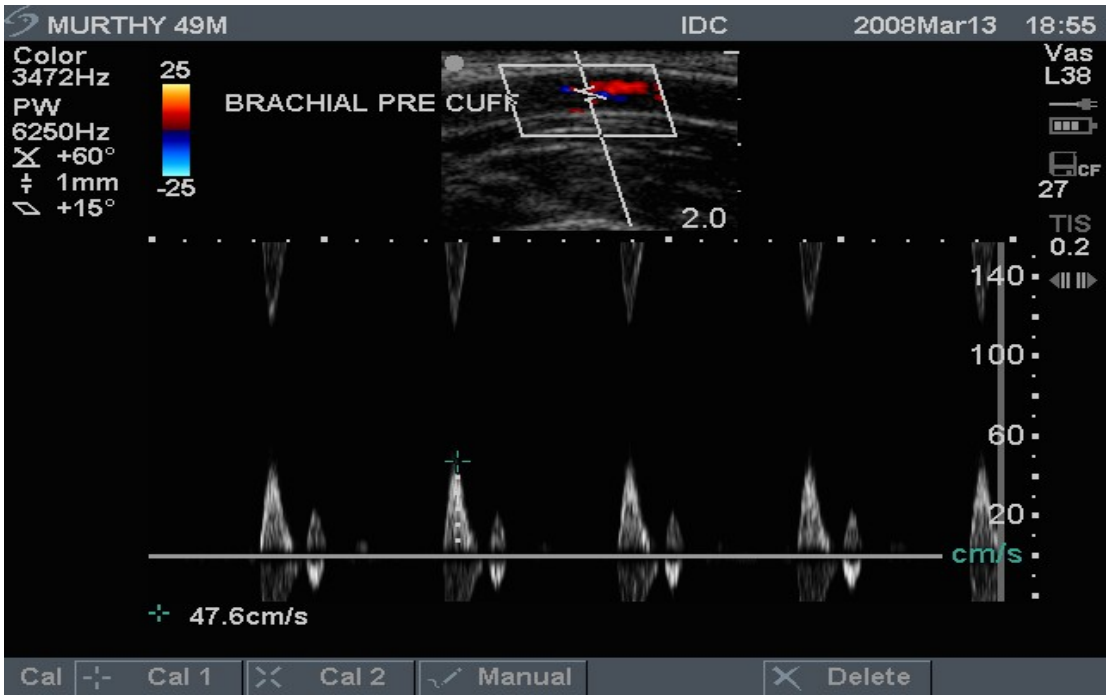


Fig.8B Patient Mr.M. Pre Cuff Baseline Brachial Artery Flow

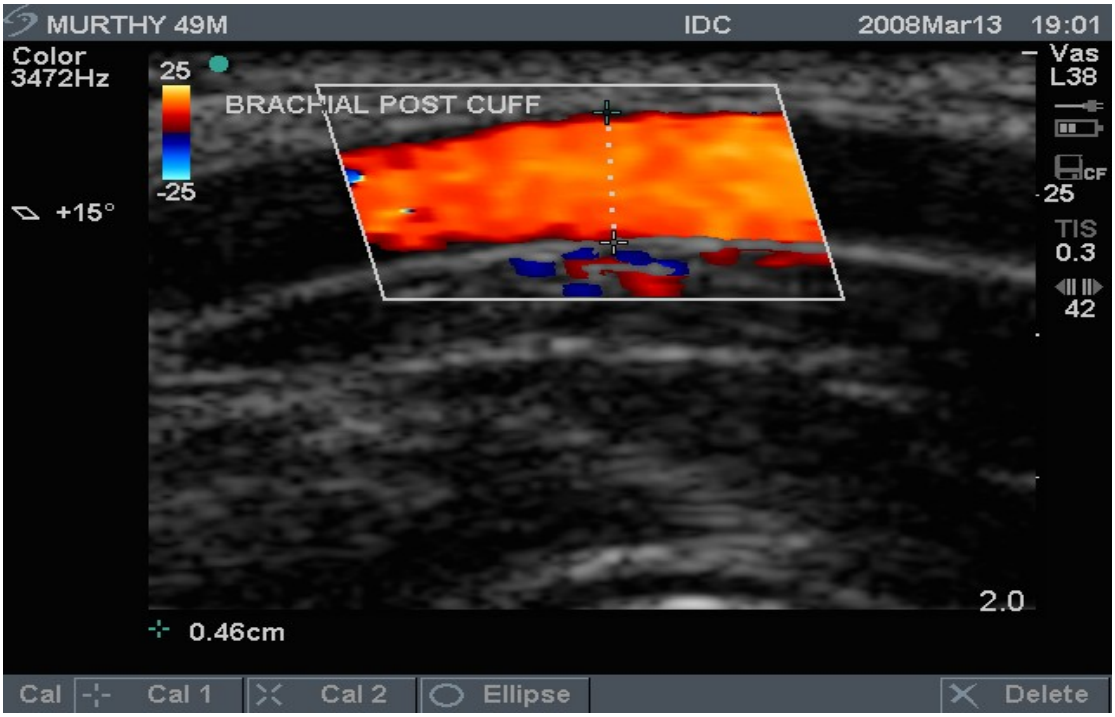


Fig.8C Patient Mr.M. Post Cuff Brachial Artery Diameter

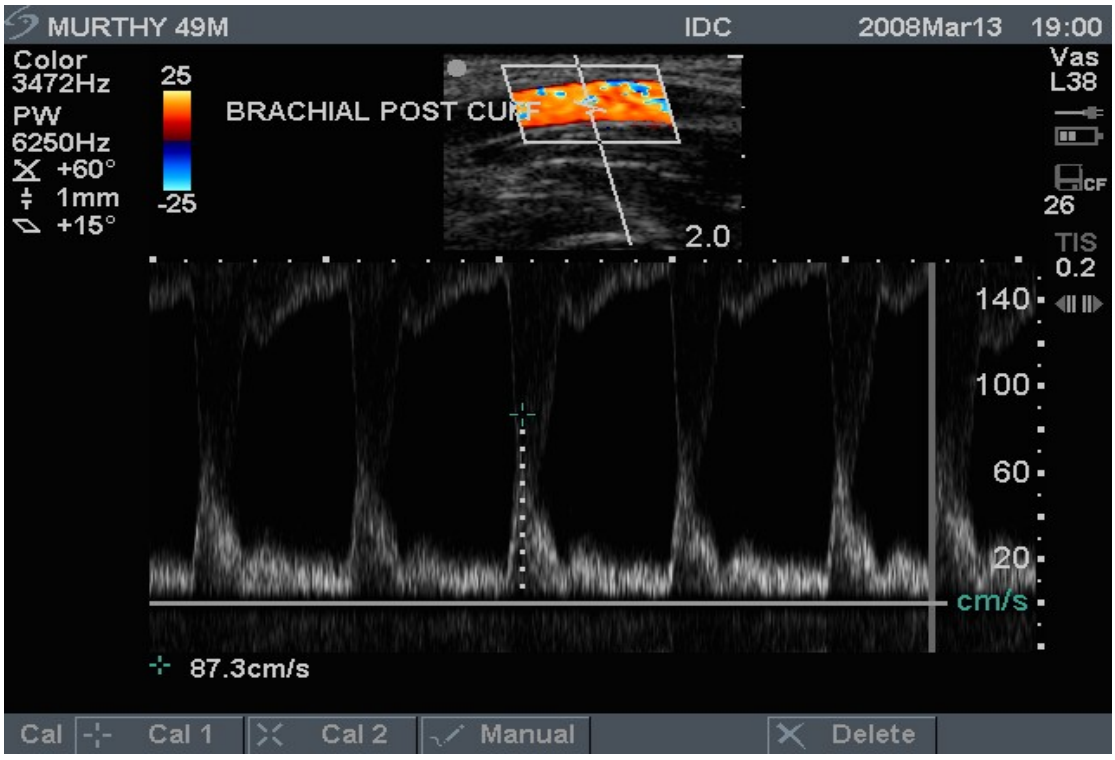
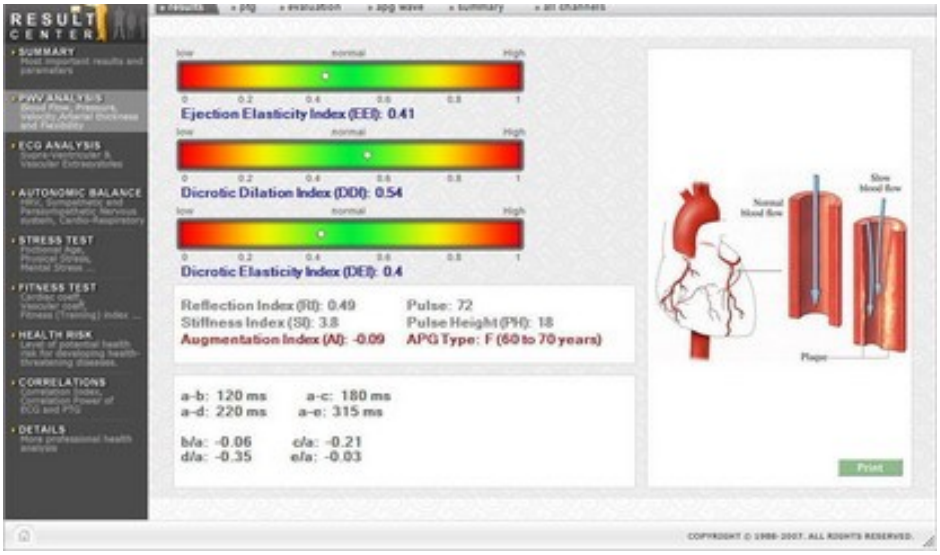


Fig.8D Patient Mr.M. Post Cuff Brachial Artery Flow



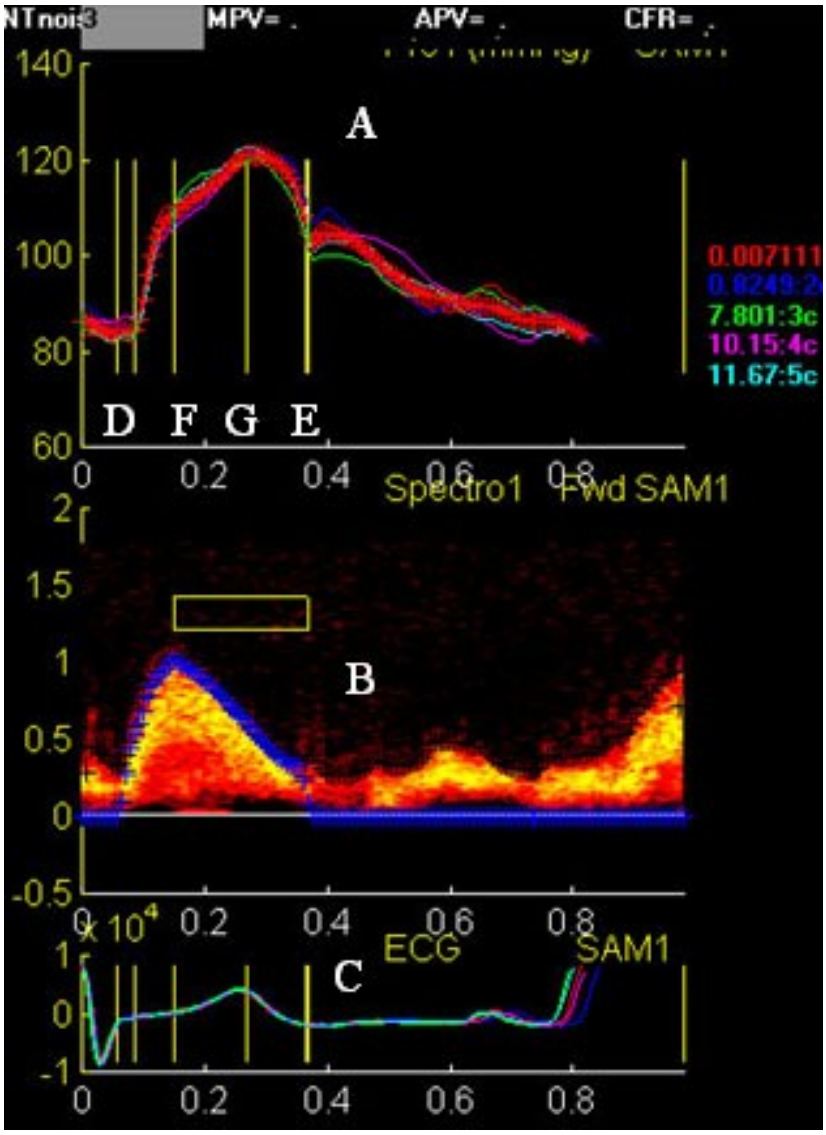


Fig.9 Patient Mr.K. Analysis of Aortic Pulse Wave Velocity